ISOLATION AND CHARACTERISATION OF NATURAL PRODUCTS FROM MEDICINALLY IMPORTANT PLANTS

SUMMARY

THESIS

SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

HASAN MAH'D HASAN MUHAISEN

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2001

SUMMARY

Plants have always been common source of medicaments either in the form of traditional preparations or as pure active principles. In a survey done by WHO it has been estimated that 80% of more than 4000 million inhabitants of the world rely chiefly on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts of their active principles. In the developed countries too plants derived drugs are important. In USA, for example, 25% of all prescriptions dispensed from community pharmacies, contain plant extracts or active principles prepared from higher plants.

It is mainly during the last 100 years that some of the active ingredients present in herbal prescriptions have been isolated and introduced into 'modern' medicine. Farnsworth et. al pointed out in their review article that there are at least 119 distinct chemical substances derived from plants that can be considered as important drugs currently in use. A few of the drugs are simple synthetic modifications of naturally occurring substances. In some instances, the natural products have now been replaced by commercially available synthetic products. Thus the drugs derived from plant still occupy an important position.

In the present studies we have carried out systematic chemical investigations of four important medicinal plants and isolated and elucidated the structures of a number of compounds, including five new constituents. These products may be helpful to other researchers who are mainly concerned with biological activity of herbal constituents.

The theoretical part of the thesis includes a critical review of the chemistry of flavonoidic as well as terpenoidic compounds and highlights the recent advances in the analytical techniques applied to their isolation and structure elucidation.

The different compounds isolated from these plants belonging to different families are as follows:

1-a Terpenoids from the leaves of Acacia tortilis (Leguminosae)

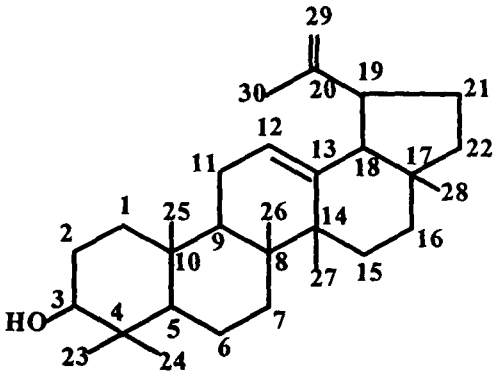
I : Lupan-3-ol,12,20-diene : (new terpenoid)

$C_{30}H_{48}O$

MW 424

M.P. 165-66°C

$[\alpha]_D^{20}$ + 24.54 ($CHCl_3$)



white shining crystals from benzene-Petrol (I)

Ac_2O/pyridine (mild) → monoacetate m.p. 152°C

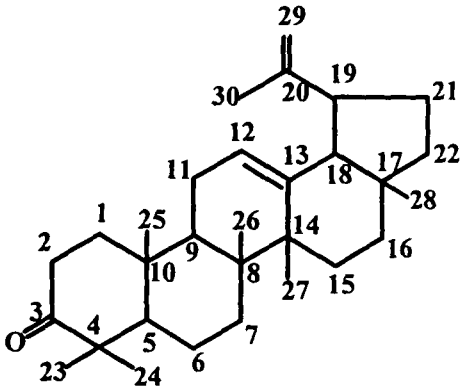
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and ^{13}C-NMR.

II : Lupan-12,20-dien-3-one : (new terpenoid)

$C_{30}H_{46}O$

MW 422

M.P. 195°C



white shining crystals from chloroform-methanol (II)

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and ^{13}C-NMR.

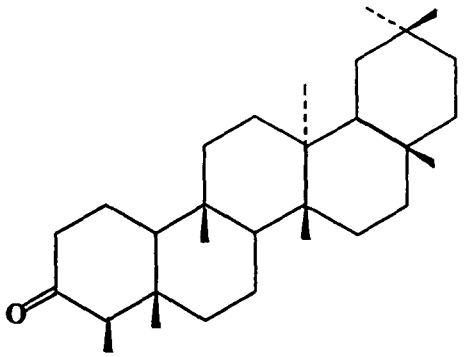
III : Friedelin:

$C_{30}H_{50}O$

MW 426

M.P. 262-64°C

$[\alpha]^{23}_{546}$ −29.4° ($CDCl_3$)



(III)

white needle crystals from chloroform-methanol.

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass.

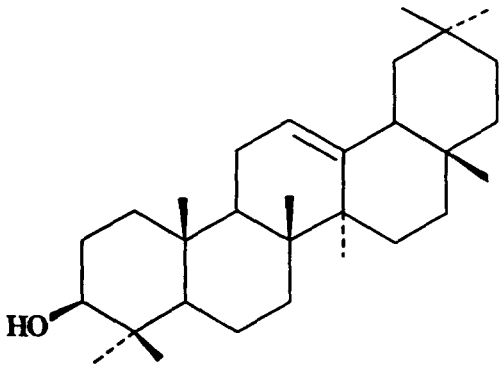
IV : β-Amyrin

$C_{30}H_{50}O$

MW 426

M.P. 198°C

$[\alpha]_D^{19}$ 88.4° ($CDCl_3$)



(IV)

white needle crystals from chloroform-methanol.

Ac_2O/pyridine (mild) → monoacetate, m.p. 241-42°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass.

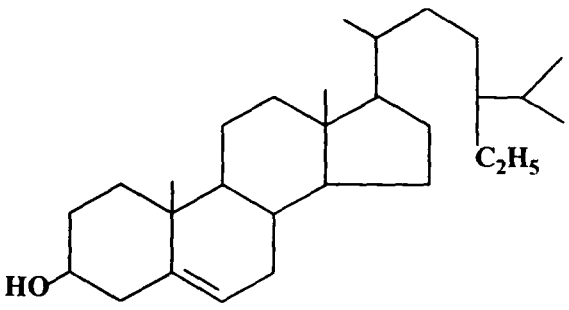
V : β-sitosterol

$C_{29}H_{50}O$

MW 414

M.P. 136-37°C

$[\alpha]_D^{22}$ −32.1° ($CDCl_3$)



(V)

white needle crystals from chloroform-methanol.

Ac_2O/pyridine (mild)→ monoacetate, m.p. 114-15°C

benzoate→ monobenzoate, m.p. 145-46°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass.

1-b Flavonoids from the leaves ofAcacia tortilis (Leguminosae)

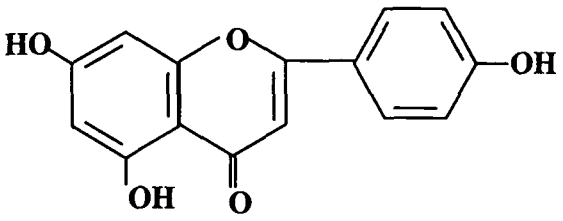
VI : 5,7,4′-trihydroxy Flavone (Apigenin)

$C_{15}H_{10}O_5$

MW 270

M.P. 352°C

Yellow crystals from benzene-acetone



(VI)

Ac_2O/pyridine (mild) → triacetate m.p. 183-84°C.

Structure elucidation is based on chemical reaction, I.R. ^{1}H-NMR, Mass and UV.

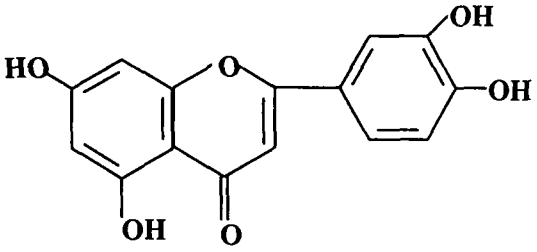
VII : 5,7,3′,4′-Tetrahydroxy flavanone (Luteolin)

$C_{15}H_{10}O_6$

MW 286

M.P. > 315°C

Yellow crystals from ethylacetate-acetone



(VII)

Ac_2O/pyridine (mild) → tetraacetate m.p. 200-01°C.

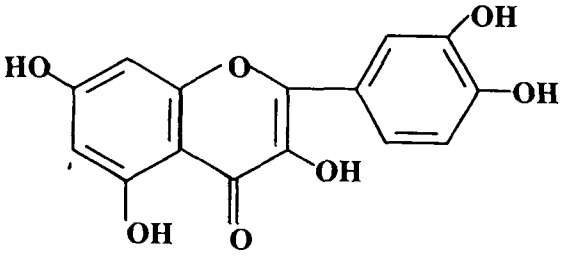
Structure elucidation is based on chemical reactions, I.R., ^{1}H-NMR, Mass and UV.

VIII : 3, 5,7,3′,4′ -Pentahydroxy flavone (Quercetin)

$C_{15}H_{10}O_7$

MW 302

M.P. 311-12°C



(VIII)

Yellow crystals from methanol

Ac_2O/pyridine (mild) → Pentaacetate m.p. 194-95°C.

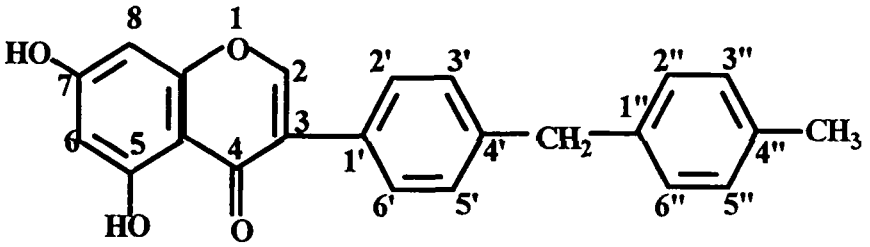
Structure elucidation is based on chemical reactions, I.R. ^{1}H-NMR, Mass and UV.

IX : 5,7-dihydroxy-4'-p-methyl benzyl isoflavone : (<u>new isoflavone</u>)

$C_{23}H_{18}O_4$

MW 358

M.P. 168°C



(IX)

Pale yellow crystals from methanol

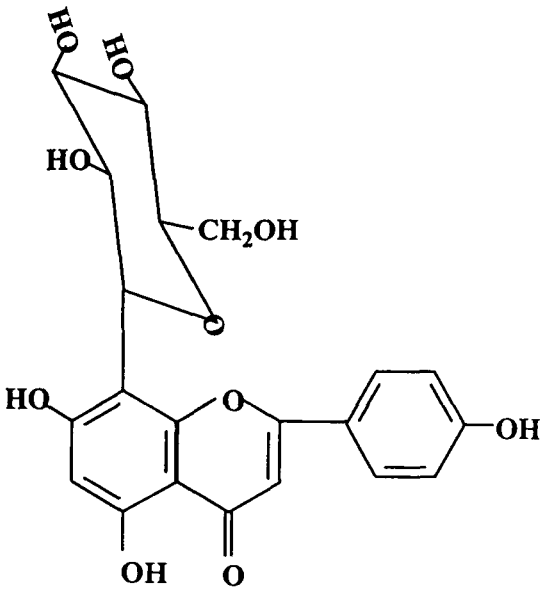
Structure elucidation is based on chemical reactions, I.R. ^{1}H-NMR, Mass and UV.

X : Vitexin

$C_{21}H_{20}O_{10}$

MW 432

M.P. 263-64°C



(X)

Yellow crystals from methanol-chloroform

Ac_2O/pyridine (mild) → heptaacetate m.p. 154-56°C.

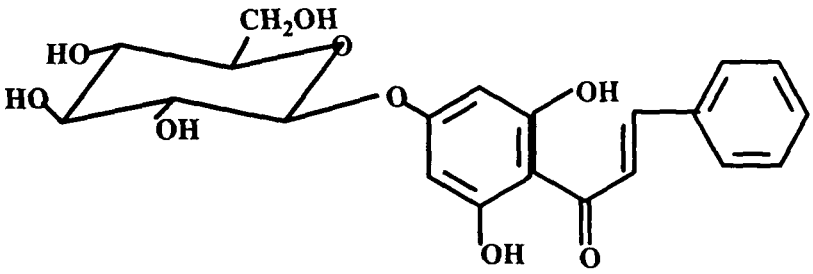
Structure elucidation is based on chemical reactions. I.R., ^{1}H-NMR, Mass and UV.

XI : 2′,6′-dihydroxy chalcone-4′-O-glucoside (new chalcone)

$C_{21}H_{22}O_9$

MW 418

M.P. >280°C



(XI)

Yellow crystals from methanol-chloroform

Ac_2O/pyridine (mild) → hexaacetate, m.p.178°C

Structure elucidation is based on chemical reactions, I.R. ^{1}H-NMR and Mass

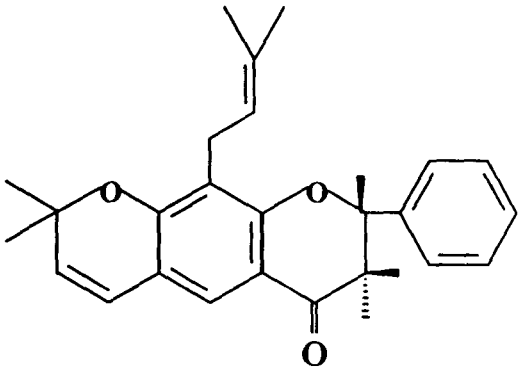
2-Flavonoids from the leaves of Lannea acida (Anacardiaceae)

I : 6,7-(2″,2″-dimethyl chromeno)-8-γ,γ-dimethylallyl flavanone

$C_{25}H_{26}O_3$

MW 374

M.P. 92°C



(I)

White needle crystals from benzene-ethylacetate

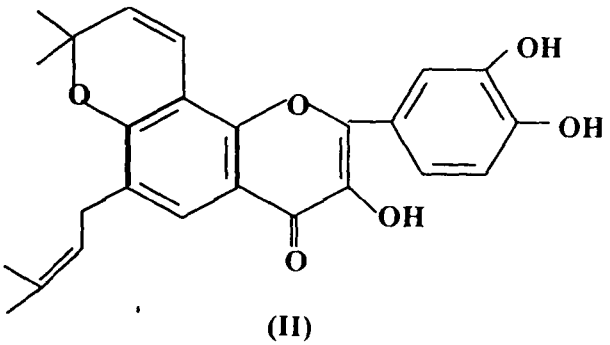
Structure elucidation is based on chemical reactions, ^{1}H-NMR and Mass

II : 3′,4′dihydroxy-7,8-(2″,2″-dimethyl chromeno)-8-γ,γ-dimethylallyl flavonol

$C_{25}H_{24}O_6$

MW 420

M.P. 165°C



(II)

Yellow needle crystals from methanol

Ac_2O/pyridine (mild) → diacetate m.p. 120-21°C

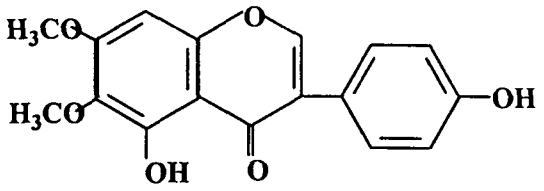
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

III : 7-methyl tectorigenin

$C_{17}H_{14}O_6$

MW 314

M.P. 236-37°C



(III)

Yellow needle crystals from methanol

Ac_2O/pyridine (mild) → diacetate m.p. 182-84°C

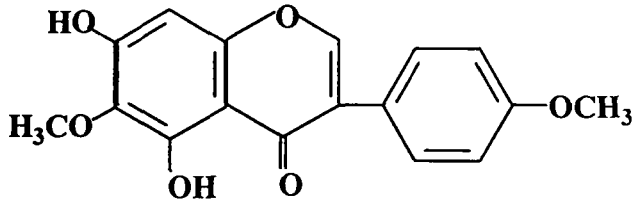
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

IV : Irisolidone

$C_{17}H_{14}O_6$

MW 314

M.P. 192-93°C



(IV)

Light yellow shining needle crystals from methanol

Ac_2O/pyridine (mild) → diacetate m.p. 162-63°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

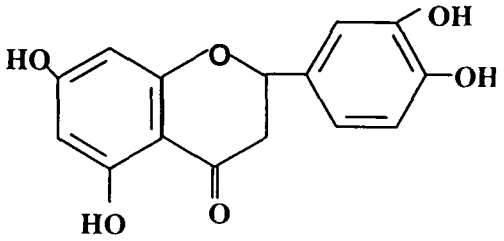
3-Flavonoids from the leaves of Viburnum cotinfolium (Caprifoliaceae)

I : 5,7,3′,4′-tetrahydroxy flavanone (Eriodictyol)

$C_{15}H_{12}O_6$

MW 288

M.P. 263°C



(I)

Yellow cubes from chloroform-methanol

Ac_2O/pyridine (mild) → tetraacetate m.p. 143-44°C.

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, UV. and Mass

II : 5,7,3′,4′-tetrahydroxy flavone (Luteolin)

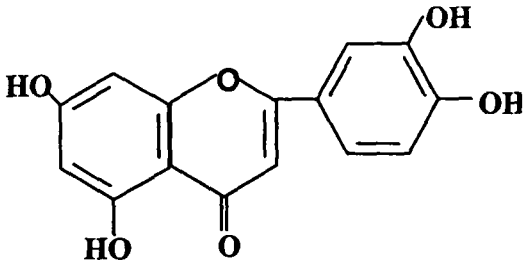
$C_{15}H_{10}O_6$

MW 286

M.P. > 320 °C

Yellow crystals from ethylacetate-acetaone

Ac_2O/pyridine (mild) → tetraacetate m.p. 200°C



(II)

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, UV. and Mass

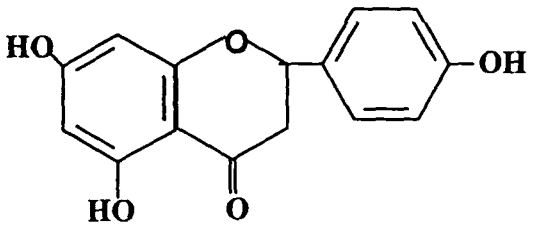
III : 5,7,4′-trihydroxy flavanone (Naringenin)

$C_{15}H_{12}O_5$

MW 272

M.P. 248-50°C

Yellow needle crystals from benzene-acetone



(III)

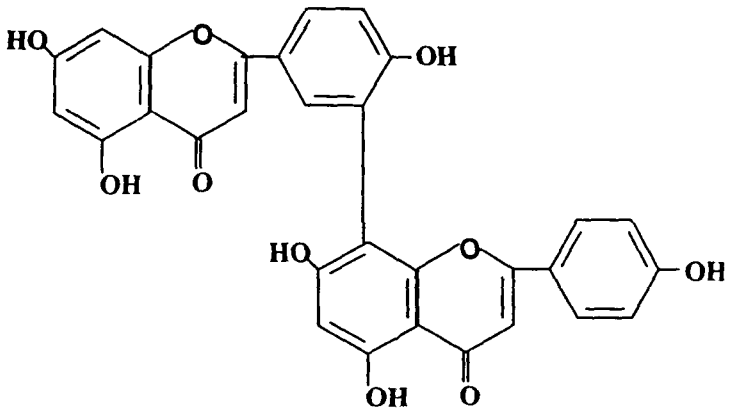
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, UV. and Mass

IV : I-4′, II-4′, I-5, II-5, I-7, II-7-hexahydroxy [I-3′, II-8] biflavone (Amentoflavone).

$C_{30}H_{18}O_{10}$

MW 538

M.P. 253-55°C



(IV)

yellow needle crystals from methanol

Ac_2O/pyridine (mild) → hexaacetate, m.p. 240-42°C

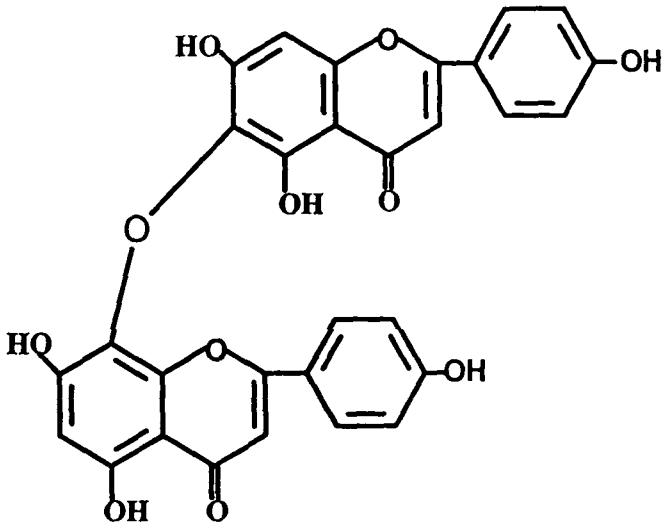
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, U.V and Mass.

V : I-5, II-5, I-7, II-7, I-4′, II-4′-hexahydroxy [6-O-8] biflavone (new biflavone).

$C_{30}H_{18}O_{11}$

MW 554

M.P. 222-23°C



(V)

Pale yellow crystals from methanol-chloroform.

Ac_2O/pyridine (mild) → hexaacetate m.p. 155°C

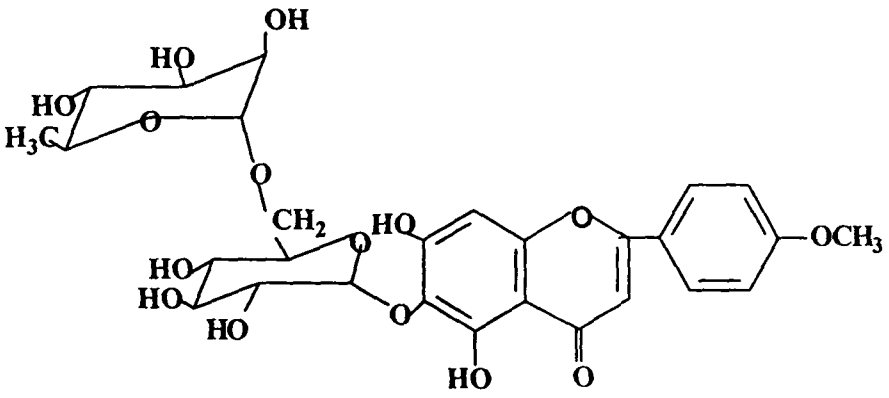
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

VI : 4′-methoxy scutellarein 6-O-rutinoside.

$C_{28}H_{32}O_{15}$

MW 608

M.P. >275°C



(VI)

Pale yellow crystals from ethyleacetate-alcohol.

Ac_2O/pyridine (mild) → octaacetate m.p. 166-68°C

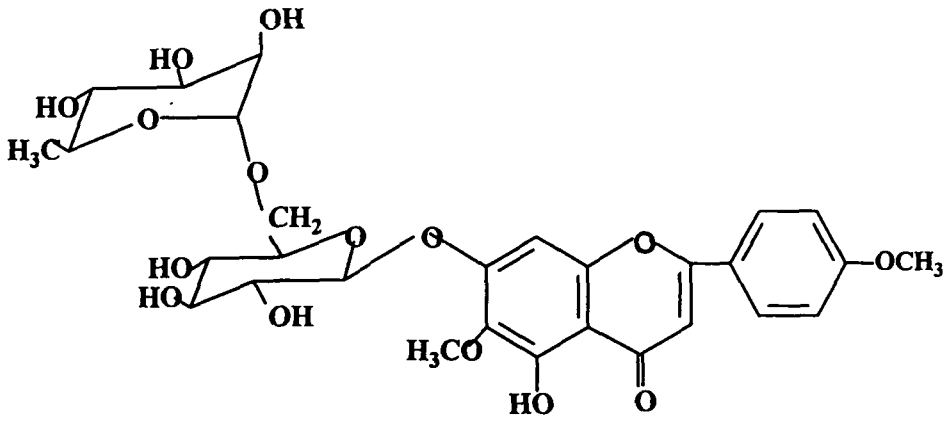
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

VII : Pectolinarigenin 7-O-rutinoside (Pectolinarin).

$C_{29}H_{34}O_{15}$

MW 622

M.P. 248-50°C



(VII)

Yellow needle crystals from ethylacetate-methanol.

Ac_2O/pyridine (mild) → heptaacetate m.p. 136-38°C

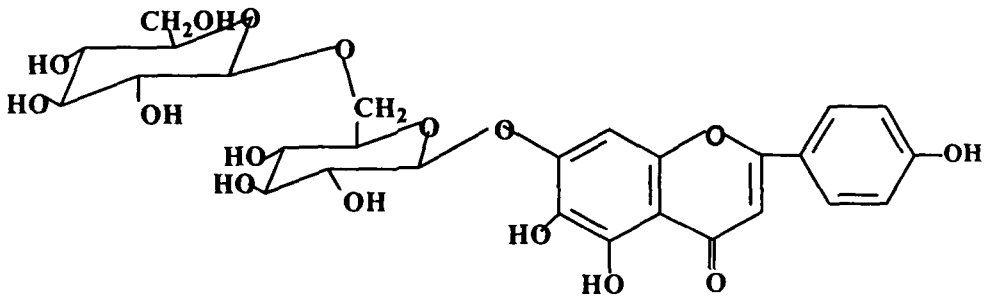
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

VIII : Scutellarein-7-diglucoside.

$C_{27}H_{30}O_{16}$

MW 610

M.P. > 300°C



(VIII)

Yellow needle crystals from benzene-methanol.

Ac_2O/pyridine (mild) → decaacetate m.p. 238-39°C

Structure elucidation is based on chemical reactions, ^{1}H-NMR, UV. and Mass.

4-a- Terpenoid from the base of leaves of Caryota urens (Palmae).

I : Triacontane : (mixture)

$C_{30}H_{62}$

MW 422

M.P. 62-67°C

Colourless crystals from carbon tetrachloride-acetone

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and GC/MS.

II : Lupeol

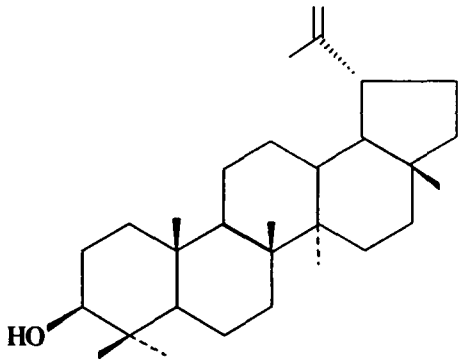
$C_{30}H_{50}O$

MW 426

M.P. 214-15°C

$[\alpha]_D^{20}$ + 23.64 ($CHCl_3$)

Solid from methanol-chloroform.



(II)

Ac_2O/pyridine (mild) → monoacetate m.p. 218-20°C

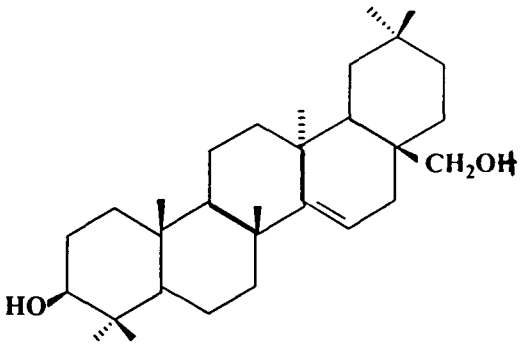
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass.

III : Myricadiol

$C_{30}H_{50}O_2$

MW 442

M.P. 259-60°C



(III)

Colourless amorphous powder from benzene-ethylacetate

Ac_2O/pyridine (mild) → diaacetate, m.p. 245-47°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass.

IV : β-Sitosterol (mixture)

$C_{29}H_{48}O$

MW 412

M.P. 159°C

$[\alpha]_D^{20}$ - 53.48 ($CHCl_3$)

White crystals solid from methanol-chloroform

Ac_2O/pyridine (mild) → monoacetate, m.p. 126°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and GC-MS.

V : Tetracosnoic acid.

$C_{24}H_{48}O_2$

MW 368

M.P. 87°C

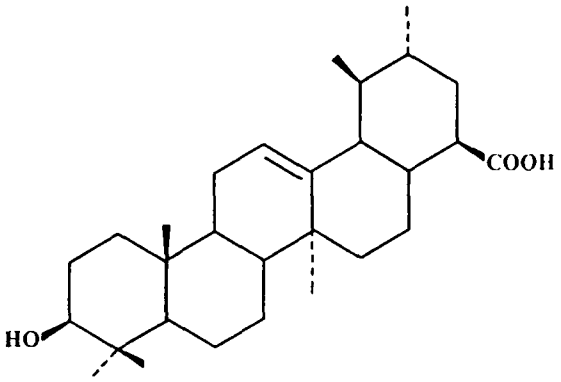
Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass.

VI : Ursolic acid

$C_{30}H_{48}O_3$

MW 456

M.P. 284-88°C



(VI)

Shining needle crystals from chloroform -methanol

Ac_2O/pyridine (mild) → monoacetate, m.p. 263-64°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR and Mass

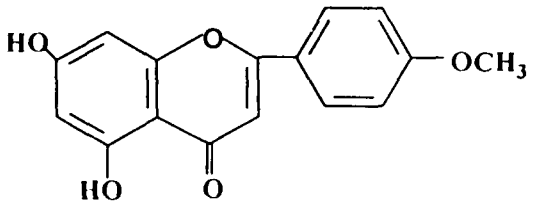
4-b- Flavonoids from the base of leaves of Caryota urens(Palmae).

VII : 5,7-dihydroxy 4'-O-methylflavone (Acacetin)

$C_{16}H_{12}O_5$

MW 284

M.P. 261-63°C



(VII)

Ac_2O pyridine (mild) → diacetate m.p. 204°C

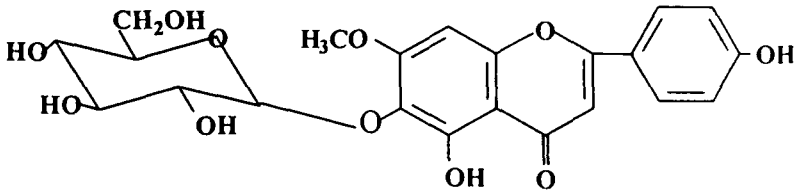
Structure elucidation is based on chemical reactions, UV and 1H-NMR.

VIII : Sorbifolin 6-glucoside

$C_{22}H_{22}O_{11}$

MW 462

M.P. > 310°C



(VIII)

Yellow needles from ethylacetate-methanol.

Ac_2O/pyridine (mild) → hexaacetate, m.p. 110-11°C

Structure elucidation is based on chemical reactions, IR, ^{1}H-NMR, Mass and UV.

ISOLATION AND CHARACTERISATION OF NATURAL PRODUCTS FROM MEDICINALLY IMPORTANT PLANTS

THESIS

SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

HASAN MAH'D HASAN MUHAISEN

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2001

DEDICATED TO MY PARENTS

Dr. M. Mushfiq
Professor of Chemistry

Tel. : 0571-400515

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

Date 3-9-01

CERTIFICATE

This is to certify that the work described in the thesis entitled ***"ISOLATION AND CHARACTERISATION OF NATURAL PRODUCTS FROM MEDICINALLY IMPORTANT PLANTS"*** is the original work carried out by **Mr. Hasan Mah'd Hasan Muhaisen** under my supervision and is suitable for submission for the award of **Ph.D.** degree in **Chemistry**.

(Prof. M. Mushfiq)

Res. B-2, M.I.G. Flats, Diggi Road, Aligarh-202001 (U. P.), INDIA Tel. 0571-401726

ACKNOWLEDGEMENTS

Praise be to Allah, the Lord of the worlds, Who says in His Glorious Book, "There has come to you from Allah a Light and a plain Book", and peace and blessings of Allah be upon the noblest of the prophets and Messengers, our Prophet Muhammad who has said, "you should insist on acquiring knowledge even if you have to travel upto China." And "Seek Knowledge from cradle to grave."

In the name of **Almighty Allah**, who favoured me to achieve this work which I thought unattainable. To whom belong might and majesty, I bring all the praises and commendation for providing me with strength during the three and half years of research which helped me to overcome the troubles and difficulties in the toilsome journey and who well-fixed me whenever I declined in vigor or vitality. Praise be to Him.

I don't find the words to express my deep sense of gratitude to my supervisor, **Prof. M. Mushifq,** Department of Chemistry, Aligarh Muslim University Aligarh, for his persistent inspiration, supervision and perspicacious suggestions throughout the course of this research work.

I express my deep sense of gratitude and indebtedness to **Prof. M. Ilyas** (Chairman), Department of Chemistry, Aligarh Muslim University, Aligarh for his scholastic guidance, inspiration and encouragement during the preparation of this thesis. His invaluable suggestions, penetrating insight, meticulous corrections, and discerning judgements taught me more than what my nineteen years of education could not. I am proud to have worked with him and want to express my humble gratefulness for all that he has done for me. Words, inadequate as they are, are my only means of expressing how beholden I am to him.

I wish to express my sincere thanks to **Dr.(Miss)Mehtab Parveen**, for all that I owe to her. The execution of this tedious work would have been very

remote,but for her unfliching support at all level and encouragement at all odd hours.

If an enterprise of this kind succeeds, it is because its author has managed to balance on the shoulders of many others, without too often falling off. Words fail me in recording my immense heartfelt gratitude to my reverend **Parents** whose blessings have always served as sheet anchor against the mundane odds and inspired me to complete my mission. I am equally beholden to my brother **Dr. Issam Muhaisen "Abu-Samer"** who has scarified a lot for me, and has been a pillar of strength and support. I am equally grateful to my brothers, **Sallah "Abu-Mah'd"** and **Mohammad.**

I duly acknowledge the kind cooperation, constant encouragement of my friends who helped me to overcome nostalgia and homesickness whenever I felt lonely. How fortunate I was to be associated with **Dr. Omer Ahmed Basudan, Dr. Wail Darwish, Dr. Ghassan Adnan, Dr. Mohammad Dabi** and my Indian as well as Mauritian intimate friends **Dr. Shafiullah, Dr. Talat Qayyum, Miss. Naazreen Moiden** and **Mr. Noosroollah Suffee** and his wife **Wahezabee Suffee.** I acknowledge their help from the core of my heart. I am grateful to **Dr. Atta Ahmad,** CDRI, Lucknow for cooperation and help in providing the spectral facilities from time to time.

I am also thankful to the chairmen, Department of chemistry for providing the necessary facilities during my research work.

Last but not the least, for neat and efficient laser-typesetting of this thesis and for punctuality as well, my special regards and thank are really extended to **Mr. Md. Hasan**, Exclusion.com, Aligarh.

HASAN MAH'D HASAN MUHAISEN

CONTENTS

CHAPTER-I

INTRODUCTION

Plants not only provided man food and fabric but also cured him from several diseases. Medication by herbs was the sole way against a lot of diseases. The early medicines of Pharaons (3,000 B.C.), the Greek (400, B.C., Hippocratics), the Roman (37 B.C., Disoscorides) as well as those of middle ages examplified by the Arab Physicians (Avicenna 980-1037, Rahzes 865-925) relied mainly on plants for therapy. In recent days attention is re-directed towards the plant drugs for less side effect~~, troubles~~. Every year a very large number of natural products are screened for their chemical, therapeutic, biological and industrial potentials. In addition to their ~~medical~~ medicinal actions, natural products are used by taxonomists for phytogenetic studies. Flavonoids and terpenoids have been used as taxonomic markers in several cases[1].

The **flavonoids**, one of the most numerous and wide spread group of the natural products are important to man not only because they contribute to plant colours but also because many members (e.g. coumestrol, phloridzin, rotenone) are physiologically active[2]. They are universally distributed among vascular plants and found practically in all parts of plants. Gabor[3] has reviewed trends in research on the pharmacodynamic effects of flavonoids, mostly rutin and its derivatives. With the extensive screening programmes of plant products for anticancer drugs,[4-7] it is not surprising that claims have been made that flavonoids may contribute to or be effective in combating certain types of cancer.[8] Numerous other physiological and biological activities have been attributed to them.[9-10] The main group of flavonoids that are well known to possess ~~f~~estrogenic activities are the isoflavones, such as genistein[11].

BIOLOGICAL ACTIVITY OF FLAVONOIDS:

It is well known that some flavonoids can act as antispasmolytic agent by relaxing smooth muscles in various parts of the mammalian body.[12] Quercetin 3-glucoside and rutin induced a concentration dependent inhibition of the spontaneous contractions of rat ileum.[13] Rutin has been reported to induce smooth muscle relaxation in various other in vitro preparation such as guinea-pig colon and rat duodenum at similar concentrations.[13]

Flavonoids may also exhibit useful antibacterial activity. Several flavonoids have been shown to have potential as hepatoprotective agents,[14] such as dihydro-

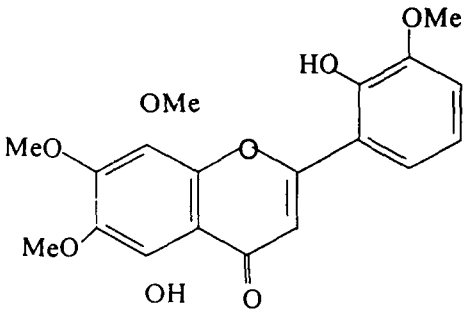
kaempferol 3-rhamnoside and 5,7,3',5'-tetrahydroxy flavanonol 3-rhamnoside, showed some hepatoprotective activity[15].

Three diprenylisoflavones, 6,8-diprenylgenistein, 6,3'-diprenylgenistein and derrisisoflavone were found to be active antifungal agents against the human pathogen, trichophyton mentagrophytes.[16]

A recent survey of the literature showed that the flavonoid field is still very popular with the chemists and their interest is increasing in isolating new flavonoids and their physiological activity. A large numbers of naturally occurring new and novel flavonoids are added to the literature every year. Few of the recently isolated flavonoids are listed for ready reference to the studies reported in the thesis.

1. **Flavone:**

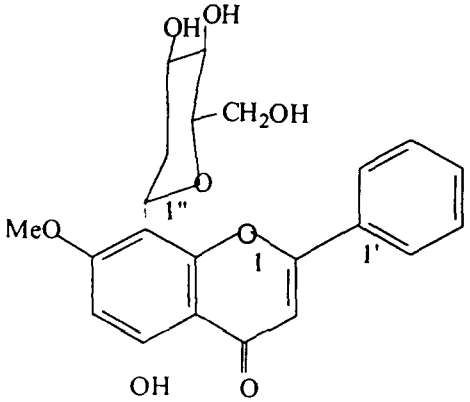
Kazutaka Nishikawa *et al.*[17] have isolated 5,2'-dihydroxy-6,7,8,3'-tetra-methoxy flavone (1), from **Scutellaria baicalensis**.



(1)

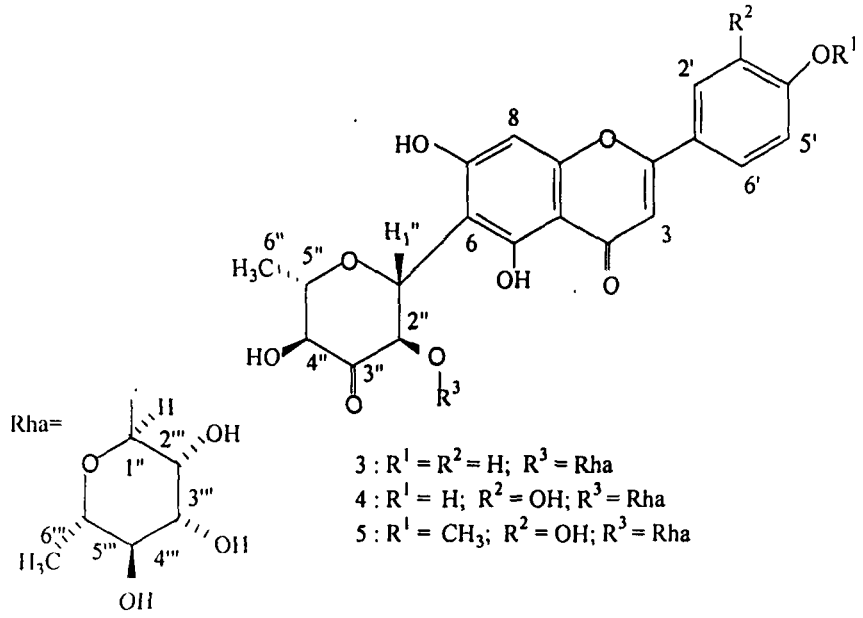
2. **C-glucosyl flavone:**

Davyson de L. Moreira *et al.*[18] have isolated kaplanin (2) from the dichloromethone fraction of the methanolic extracts of leaves of **Pipes ihotzkyanum**.



(2)

Tsutomu Hatano *et al.*[19] have isolated three C-glycosidic flavonoids, cassiaoccidentalin A-C (3-5) from **Cassia occidentalis**.



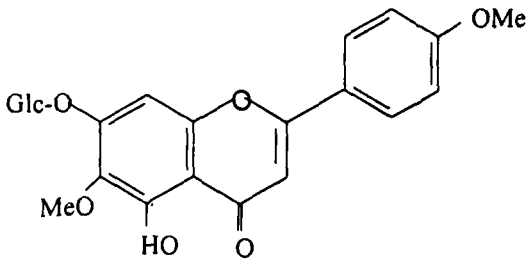
3 : $R^1 = R^2 = H$; R^3 = Rha

4 : R^1 = H; R^2 = OH; R^3 = Rha

5 : $R^1 = CH_3$; R^2 = OH; R^3 = Rha

3. Flavone glycosides:

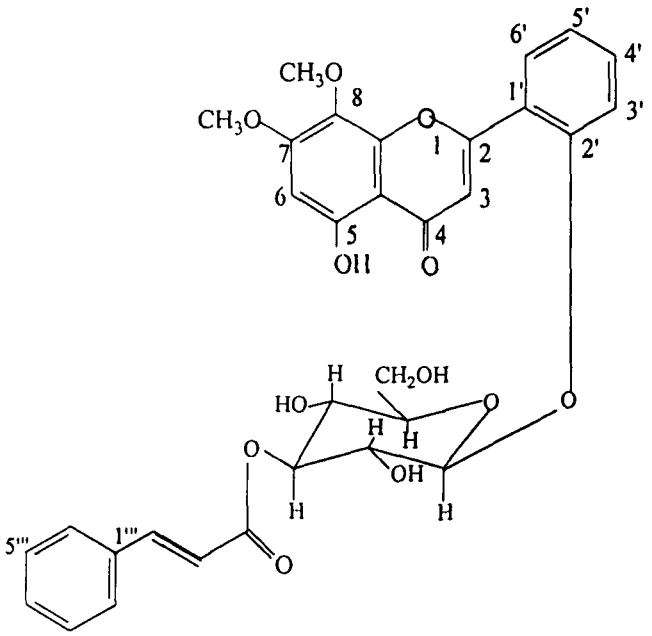
Salwa F. Farag *et al.*[20] have isolated new flavone glycoside, identified as 6,4'-dimethoxy-5-hydroxy flavone 7-O-β-D-glucopyranoside (6) from rhizomes **Iris carthaliniae**.



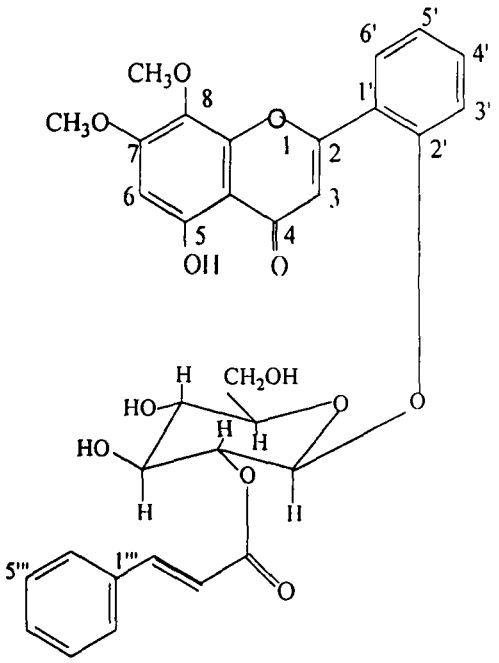
(6)

4. Acylated Flavone glucosides A.F.G.:

A.G Damu *et al.*[21] have isolated two new A.F.G skullcapflavone I 2'-O-β-D-(3"-E-cinnamonyl) glucopyranoside (7) and skullcapflavone I 2'-O-β-D (2"-E, cinnamonyl) glucopyranoside (8) from the whole plant of **Andrographis serpyllifolia**.



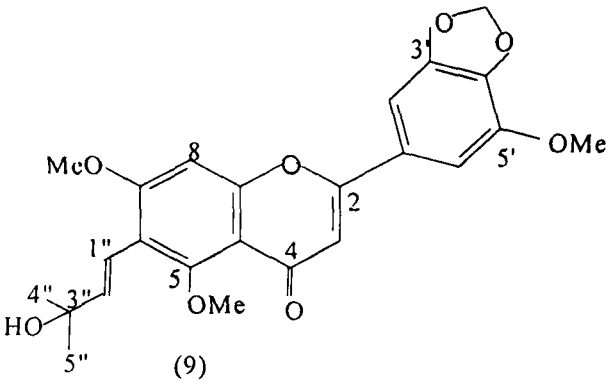
(7)



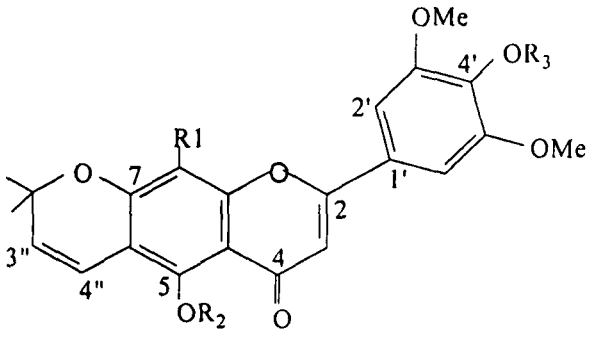
(8)

5. Prenylated flavones:

Jose I. de Souze et al.[22] have isolated three new prenylated flavones, identified as 5,7,5'-trimethoxy-6-(3"-hydroxy, 3"-methyl-trans-but-1"-enyl)-3',4'-methylene-dioxy-flavone (9), 5,4' dihydroxy-3',5'-dimethoxy-6-7-(2",2"-dimethyl pyran)-flavone (10) and 5,4'- dihydroxy-8,3',5'-trimethoxy-6,7-(2",2"-dimethylpyran)-flavone (11) from the aerial parts of **Neoraputia paraensis**.



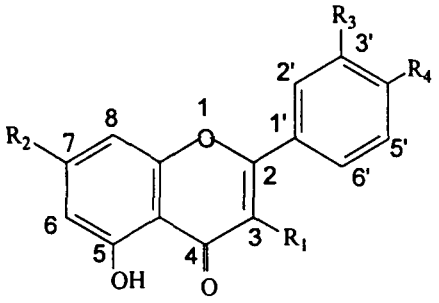
(9)



10 : R_1 = H; R_2 = H; R_3 = H
11 : R_1 = OMe; R_2 = H; R_3 = H

6. Flavonoid sulfates:

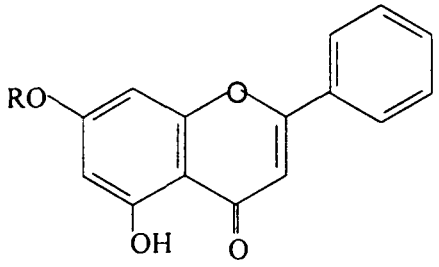
Petra Mann et al.[23] have isolated three novel compounds, quercetin 7-methylether-3,3'-disulfate (12), 3,7-dimethylether-4'-sulfate (13), and quercetin 3',4',7-trimethylether-3-sulfate (14) from different **Argyreia & Ipomoea species**.



12 : $R_1 = OSO_3Na$; $R_2 = OCH_3$; $R_3 = OSO_3Na$; $R_4 = OH$
13 : $R_1 = CH_3$; $R_2 = OCH_3$; $R_3 = OH$; $R_4 = OSO_3Na$
14: $R_1 = OSO_3Na$; $R_2 = OCH_3$; $R_3 = OCH_3$; $R_4 = OCH_3$

7. Flavonoid glucuronides:

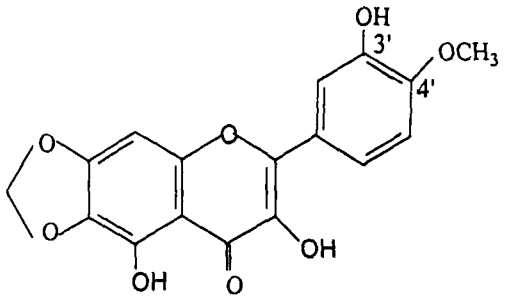
Ying Huang *et al.*[24] have isolated three flavonoid glucuronides, 7-O-β-glucuronide of apigenin and Luteolin and apigenin 7-O-β-(2"-O-α-rhamnosyl) glucuronide (15) from **Picria fel-terrae**.



(15) : R = (2"-O-α-rhamnosyl)-β-glucuronyl

8. A methylene dioxy flavonol:

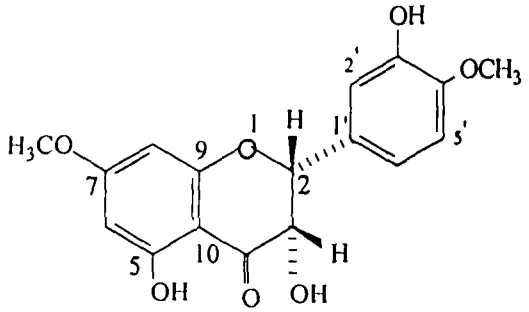
Eliane O. Ferreira *et al.*[25] have isolated 3,5,3'-trihydroxy-4'-methoxy-6,7-methylene dioxy flavonee (16) from arrial part of **Blutaparon portulacuides**.



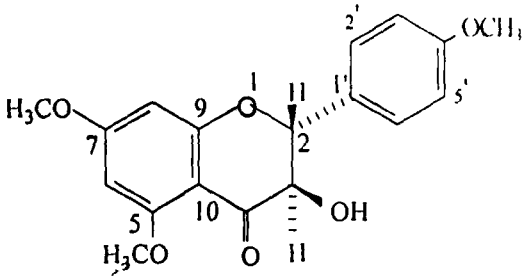
(16)

9. Dihydroflavonols:

~~Md. Tofazzal~~ Islam *et al.*[26] have isolated (2R, 3S)-(+)-3', 5-dihydroxy-4',7-dimethoxy dihrydroflavonol (17) and (2R, 3R)- (+)-4',5,7-trimethoxy dihydro-flavonol (18) from the stem bark of **Lannea coromandelica**.



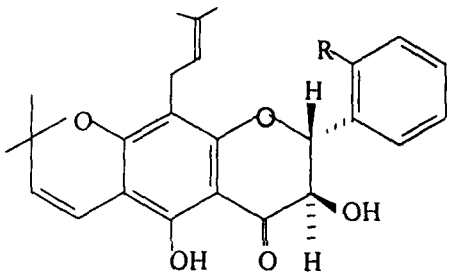
(17)



(18)

where is the third one?

Dagoberto Alavez-Solano *et al.*[30] have isolated three 3-hydroxyflavanones, jayacanol (19) and mundulino (20) from roots of **Lonchocarpus oaxacensis**.

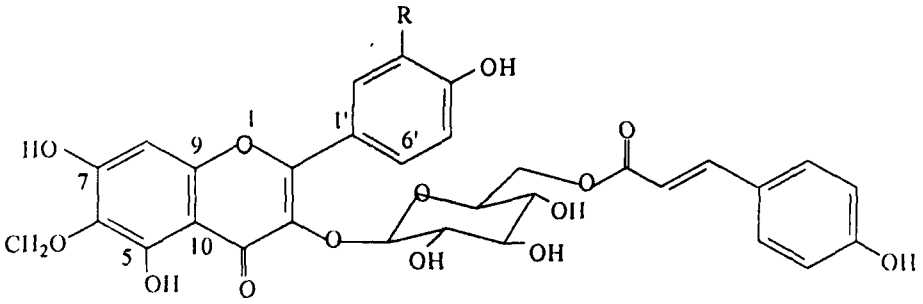


19 R = OH
20 R = H

?

10. Acyl glucosylated flavonols: A.G.F

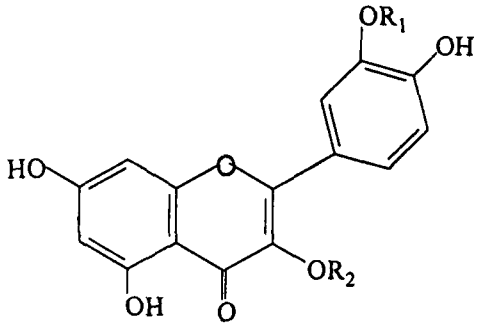
~~Fabio D.P. de~~ Andrade *et al.*[27] have isolated two new A.G.F. 6-methoxy kaempferol-3-β-D-6" (P-coumaroyl) glucopyranoside (21) and 6-methoxy quercetin-3-O-β-D-6" (P-coumaroyl) glucopyranoside (22) from **Paepalanthus polyanthu, P. robustus, P. ramosus, P. hilairci and P. denudatus .**



21 : R = OH
22 : R = H

11. Flavonol glycosides:

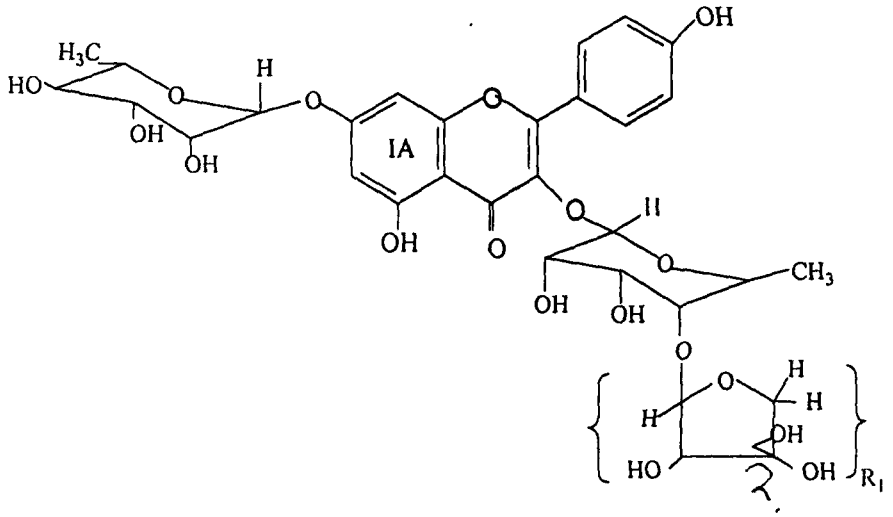
~~Mona-Antonia~~ Beck *et al.*[28] have isolated two new flavonol 3-O-glycosides which were identified as, quercetin 3-O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (23) and isorhamnetin-3-O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (24), from aerial parts of **Eschscholtzia california cham**.



23 : R_1 = H ; R_2 = α-L -Rha (1-4)-α-L-Rha (1-6) -β-D-Gl
24 : R_1 = CH_3; R_2 α-L -Rha (1-4)-α-L-Rha (1-6) -β-D-Gl

~~Ahmed A.~~ Gohar *et al.*[29] have isolated two new triglycosides, Kaempferol-3-O-[(4-β-D-apiofuranosyl)-α-L-rhamnopyranoside]-7-O-α-L-rhamnopyranoside (25) and

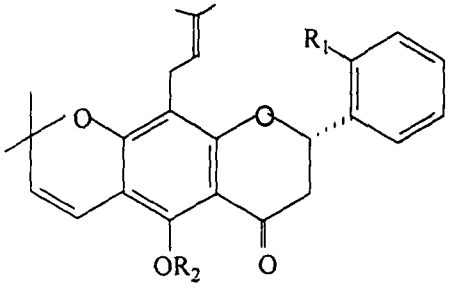
Kaempferol-3-O-[(4-β-D-xylopyranosyl)-α-L-rhamnopyrnoside]-7-O-α-L-rhamno-pyranoside (26) from **Chenopodium murale**.



25 : R_1 = Api.
26 : R_1 = Xyl.

12. Flavanones:

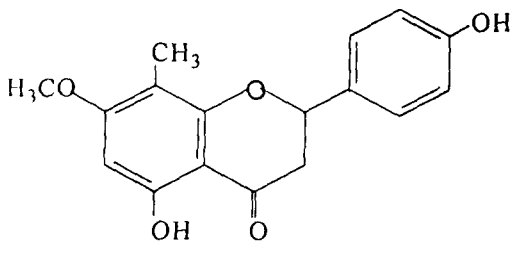
~~Dagoberto~~ Alavez-Solano *et al.*[30] have isolated two flavanones, mundulin (27) and minimiflorin (28) from roots of **Lonchocarpus oaxacensis**.



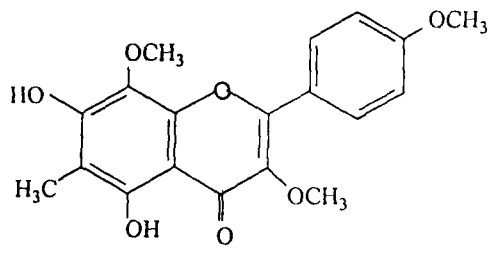
27 : R_1 = H; R_2 = H
28 : R_1 = OH ; R_2 = H

13. C-Methyl-flavonoids:

~~Eckhard~~ Wollenweber *et al.*[31] have identified two C-methyl-flavonoid aglycones as 5,4'-dihydroxy-8-C-methyl-7-methoxy flavanone (29) and 5,7-dihydroxy-3,8,4'-trimethoxy-6-C-methylfavone (30) from the leaves waxes of some **Myrtaceae**.



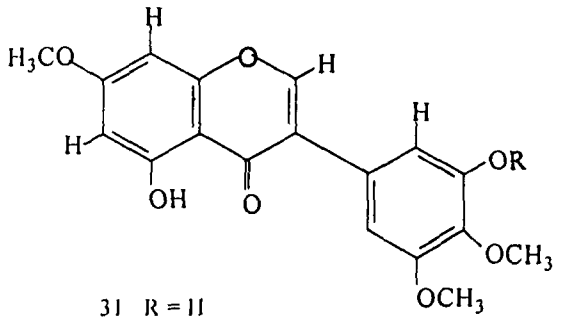
(29)



(30)

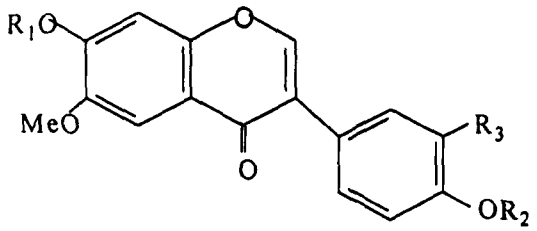
14. Isoflavone:

~~F.N.~~ Ngounou *et al.*[32] have isolated isoflavones pentandrin (31) and pent20audrm glycoside (32), from stem bark of **Ceiba pentandra**.



31 R = H
32 R = Glucose

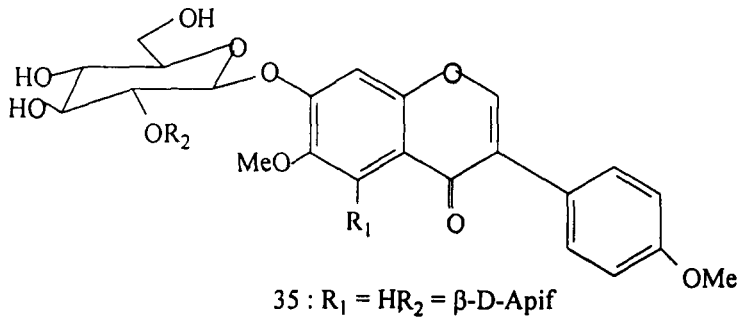
~~Salwa F.~~ Farag *et al.*[33] have reported two new isoflavonoids, tectorigenin 4'-O-β-D-glucopyranosyl (1→6)-β-D-glucopyrnoside (33), iristectorigenin B_7-β-D-Luco-pyranosly (1→6)-β-D-glucopyranoside (34) from rhizomes **Iris carthaliniac**.



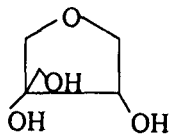
33 : R_1 = H ; R_2 = Gl-Gl; R_3 = H
34 : R_1 = Gl-Gl; R_2 = H ; R_3 = OMe

15. Isoflavone glycosides:

~~Joao B.F.~~ Tostes *et al.*[34] have isolated Afromosin 7-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (35) from the seeds of **Centrosema pubescens**.



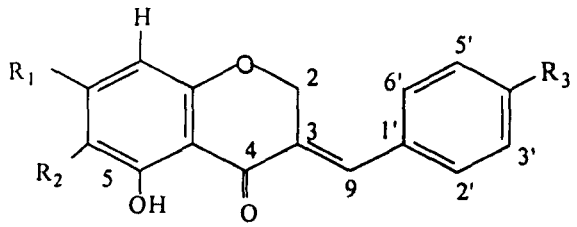
35 : R_1 = H R_2 = β-D-Apif



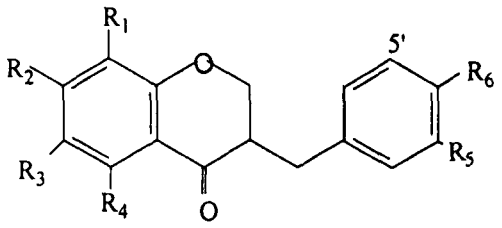
R_2 = β-D-Apif

16. Homoisolfavonoids:

Alfonse Silayo *et al.*[35] have isolated nine new homoisoflavonoids 3-(4-methoxy benzylidene)-5,7-dihydroxy-6-methoxy chroman-4-one (36), 3-(4-methoxy benzylidene)-5,7-hydroxy-7-methoxy chroman-4-one (37), 3-(4-methoxy benzyl)-5,7-dimethoxy chroman-4-one (38), 3-(4-hydroxy 3-methoxybenzyl)-5-hydroxy-7-methoxy chroman-4-one (39), 3-(4-hydroxy 3-methoxy benzyl)-5-hydroxy-6,7-dimethoxy chroman-4-one (40), 3-(3, 4-dimethoxy benzyl)-5,7-dihydroxy chroman-4-one (41), 3-(4-methoxybenzyl)-6-hydroxy-5,7-dimethoxy chroman-4-one (42), 3-(4-hydroxybenzyl)-5,6,7-trimethoxy chroman-4-one (43), 3-(4-methoxybenzyl)-8-hydroxy-5,7-dimethoxy chroman-4-one (44), from bulbs of **Scilla nervosa**.



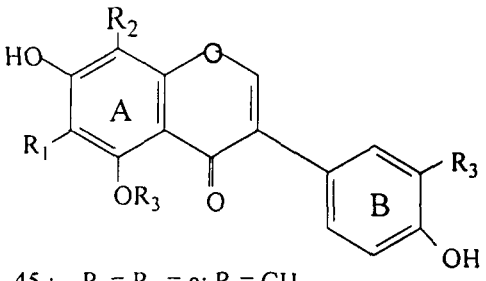
36 : R_1 = HO ; R_2 = MeO; R_3 = MeO
37 : R_1 = HO; R_2 = H; R_3 = HO



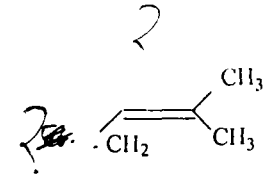
	R_1	R_2	R_3	R_4	R_5	R_6
38:	H	MeO	H	MeO	H	MeO
39:	H	MeO	H	HO	MeO	HO
40:	H	MeO	MeO	HO	MeO	HO
41:	H	HO	H	HO	MeO	MeO
42:	H	MeO	HO	MeO	H	MeO
43:	H	MeO	MeO	MeO	H	HO
44:	OH	MeO	H	MeO	H	MeO

17. Diprenyl isoflavones:

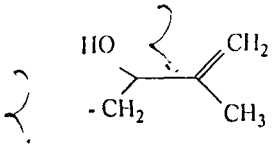
Toshikazu Sekine *et al.*[36] have isolated six new diprenyl isolafavones named dirrisioflavones A-F (45-50), from stem of **Derris scandens.**



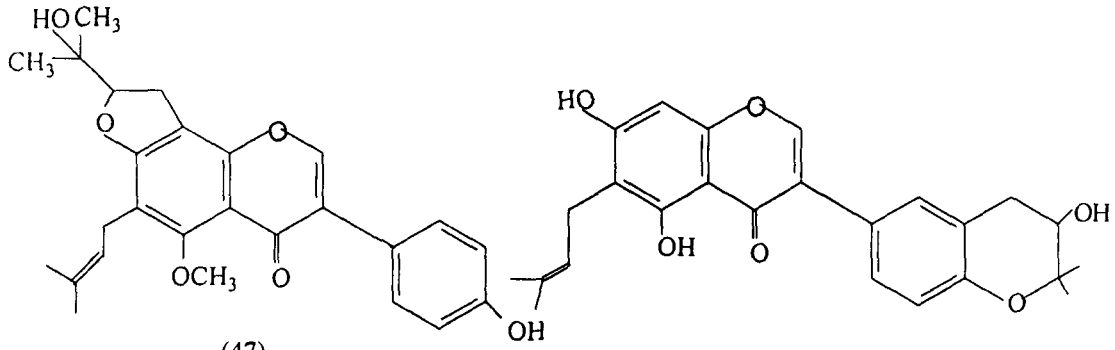
45 : $R_1 = R_2 = a$; $R = CH_3$
46 : $R_1 = a$; $R_2 = H$; $R_3 = b$; $R = H$
48 : $R_1 = b$; $R_2 = a$; $R = CH_3$
49 : $R_1 = a$; $R_2 = b$; $R = CH_3$



(a)



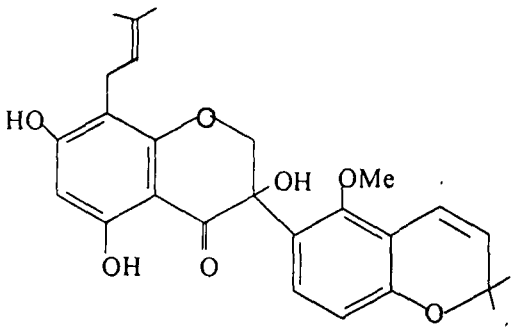
(b)



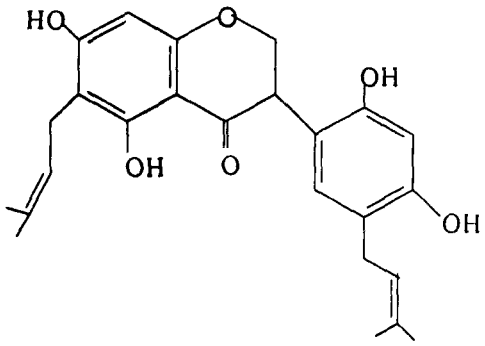
(47) (50)

18. Isoflavanones:

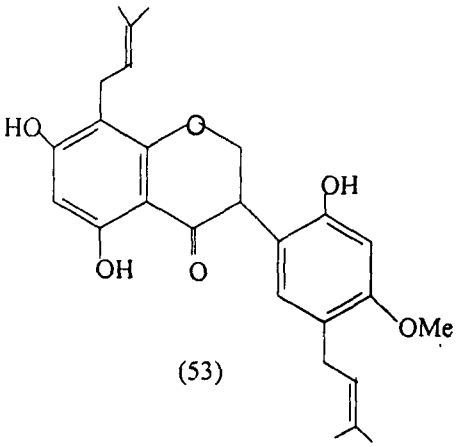
Yoshiaki Skirataki *et al.*[37] have isolated four new isoflavanones, tetrapterol F-I (51-54) from roots of **Sophora tetraptera**.



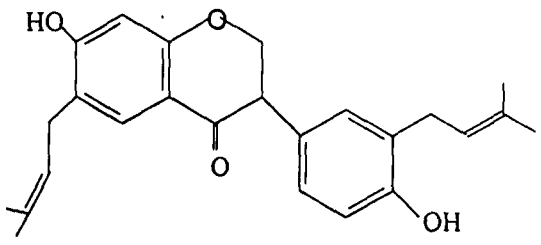
(51)



(52)



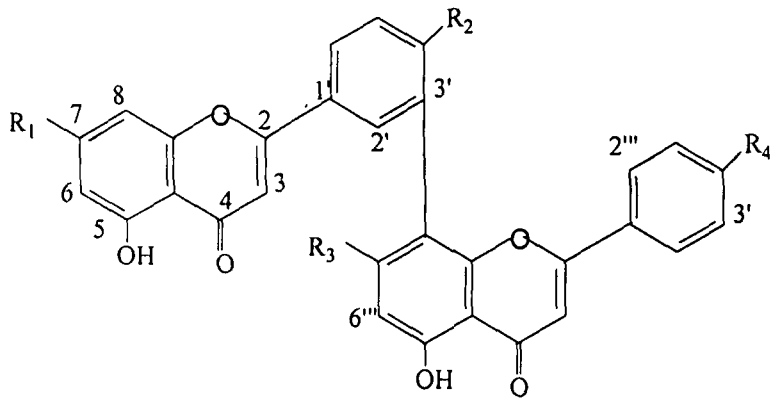
(53)



(54)

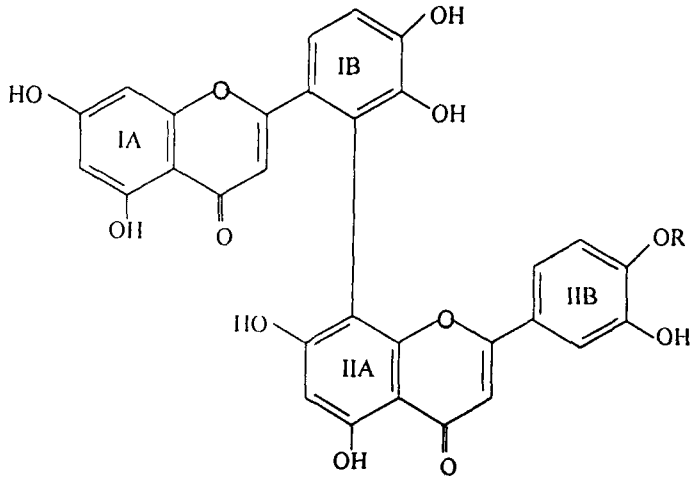
19. Biflavones:

Fabiana N Fonseca *et al.*[38] have isolated three biflavones 7,4',7"-tri-O-methylamentoflavone (55), 7,4',4"'-tri-O-methylamentoflavone (56), and 4',4"'-di-O-methylamentoflavone (57), from seedling stem of **Araucaria angustifolia**.



55 : $R_1 = R_2$; R_3 = OMe; R_4 =H
56 : $R_1 = R_2$; R_4 = OMe; R_3 = OH
57 : $R_1 = R_3$ = OH; $R_2 = R_4$ = OMe

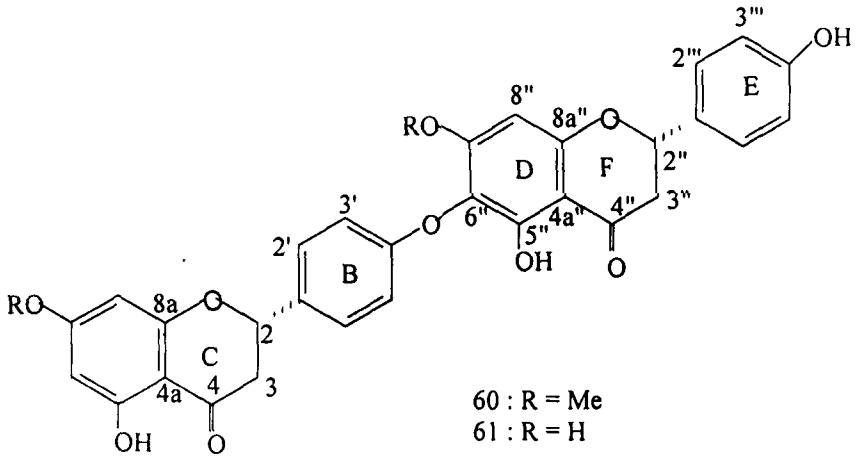
Elke Brin Kmeier *et al.*[39] have isolated two hitherto unknown biflavonoids, which have been identified as philonotisoflavone-4'–methyl (58) and 2",3"-Dihydrophilnotisflavone (59) from the moss of **Mnium hornum**.



58 : R = CH_3
59 : R = H

20. Biflavanone:

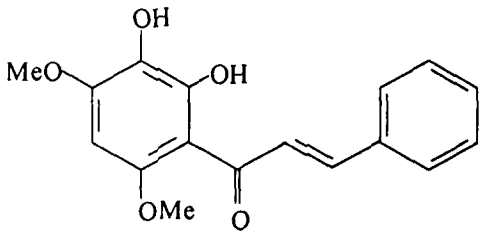
Jayaprakasam *et al.*[40] have isolated a new biflavanone, 7,7"-di-O-methyl tetrahydrohinokiflavone (60) together with tetrahydrohinokiflavone (61) from stem of **Cycas beddomi**.



60 : R = Me
61 : R = H

21. Chalcone:

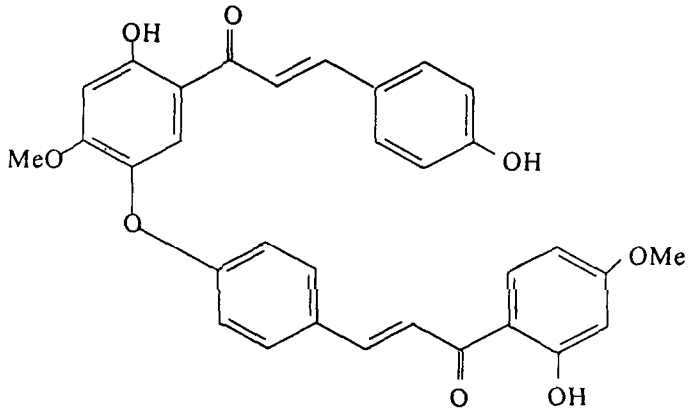
Kan Chantra Promna *et al.*[41] have isolated a chalcone, 2',3'-dihydroxy-4',6'-dimethoxy chalcone (62) from leaves of **Uvaria duleis**.



(62)

22. Bicholcone:

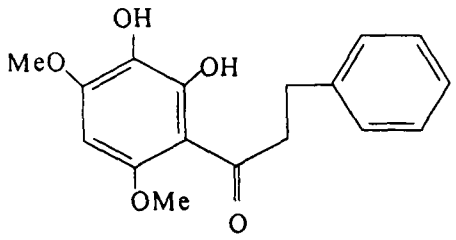
Ishmael B. Masesane *et al.*[42] have isolated a novel bichalcone, rhuschalcone (63) from twigs of **Rhus pyroldes**.



(63)

23. Dihydrochalcone:

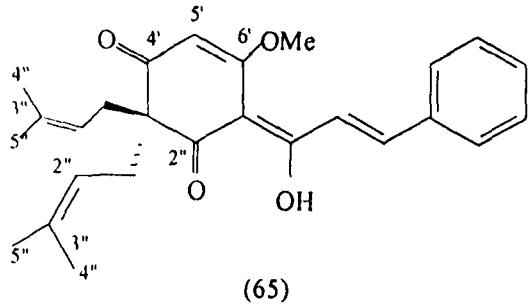
~~Kan~~-chantrapromma *et al.*[43] have isolated a new dihydrochalcone (64) from leaves of **Uvaria duleis**.



(64)

24. Enolchalcone:

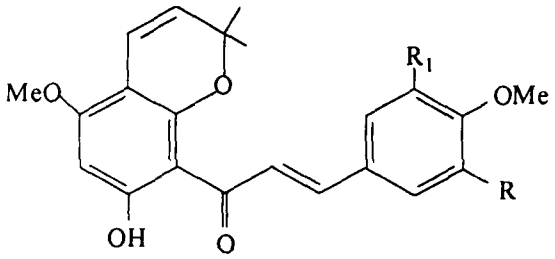
~~Cesar C~~. Andrci *et al.*[44] have isolated 3',3'-di-(γ-γ-dimethylallyl)-2',4'-di-oxo-enolchalcone (tunicatachalcone) (65) from root of **Tephrosia tunicata**.



(65)

25. Pyrano chalcones:

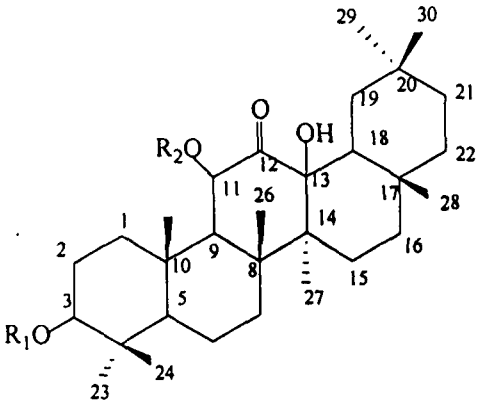
~~Daniela M~~. Tomazela *et al.*[45] have isolated 2'-hydroxy-4,4'dimethoxy-5',6'-(2", 2"-dimethyl pyrano) chalcone (66) and 2'-hydroxy-3,4,4'-trimethoxy-5',6'-(2",2"-dimethyl pyrano) chalcone (67) from **Neoraputia magnifica**.



66 : R = R_1 = H
67 : R = OMe ; R_1 = H

26. Oleanane triterpenoids: (OT)

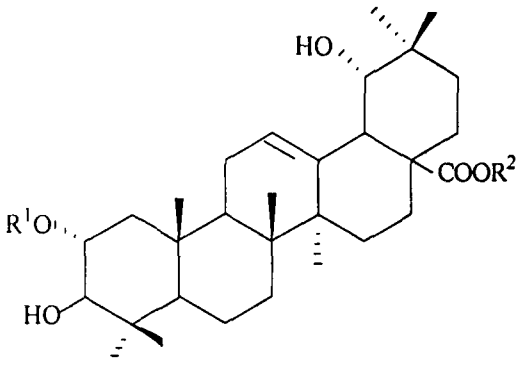
~~H.M.T.Ba~~ Herath *et al.*[46] have isolated two new O.T., 3β-acetoxy-11α, 13β-dihydroxyoleane-12-one (68) and 3β, 11α-diacetoxy-13β-hydroxyoleane-12-one (69) from **Gordonia ceylanica**.



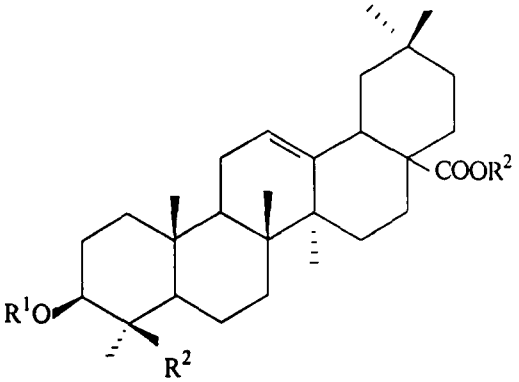
68 : $R_1 = COCH_3$; $R_2 = H$
69 : $R_1 = R_2 = COCH_3$

27. Triterpene saponins: (T.S.)

~~Salvatore de~~ Rosa *et al.*[47] have isolated three new T.S. named rivaloside C-E-(70-72) from **Galium rivale**.



70 : R^1 = AC; R^2 = Gl (1→ 6) Gl (C)
71 : R^1 = H; R^2 = Gl (1 →6) Gl (D)



72 : R^1 = GlUA; R^2 = CH_2OH; R^3 = Glc (E)

In the present study, we have tried to carry out systematic chemical investigations of some important medicinal plants with a view to characterize their chemical components preferably flavonoids, which could be the starting point for chemists who are mainly concerned with pharmacological and clinical aspects of the herbal drugs.

Since mainly the spectroscopic techniques, **uv, ir, ^{1}H-nmr, ^{13}C-nmr** and **mass** have been used in the identification and structure elucidation of the products isolated from different plants during the course of this work, a short review of each technique has been briefly discussed:

1. Infra-Red Spectroscopy:

The **ir** spectrum in practice, ~~plays an important role and~~ offers the first clue to the nature of the compound. It provides a valuable information of functional groups in a molecule. In the case of flavonoids, **ir** measurements are helpful in providing evidence for the presence of (a) pyrone ring (b) chelated hydroxyl groups and (c) the gem dimethyl grouping. The substitution pattern of the benzene ring can be inferred from bands in the 690-800 cm^{-1} region. Such evidence is helpful in distinguishing between flavnoids and coumarins. **IR** spectroscopy has mostly been used to adduce corroborative evidence.

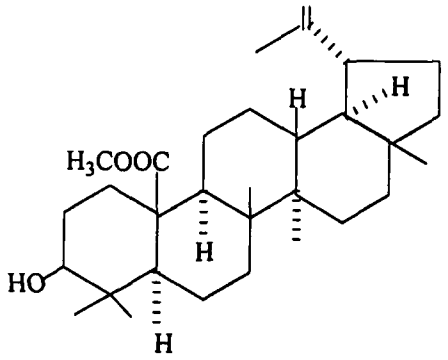
The **ir** spectra of flavones show the carbonyl band at 1660 cm^{-1} [48] owing to the conjugation with olefinic double bond. Introduction of a hydroxyl group at 5-position does not alter the band position appreciably. Luteolin and apigenin show the carbonyl bands at 1655 and 1650 cm^{-1} respectively.[49]

The **ir** spectrum of unsubstituted flavanone shows the carbonyl absorption at 1680 cm^{-1}, the standard value for aromatic ketones. The shift of carbonyl band to 1620 cm^{-1} in 5-OH flavanone is largely due to chelation. Consequently, methylation of the 5-OH produces only a small frequency shift. The existence of chelation is, however, clearly demonstrated by the absence of the hydroxyl bands at the usual position in 5-hydroxy compounds. Apparently it comes to lie in the –OH stretching region and is thus obliterated. A similar high frequency shift of 4'-substituted flavanone is attributed to intermolecular hydrogen bonding[50]. The **ir** spectra of isoflavones are similar to those of flavones. Another interesting feature of the **ir**

spectra of flavones is that the carbonyl frequency is independent of the substitution pattern in ring-A & B and is effected only by the introduction of a hydroxyl at 3-position[51].

The infrared spectra of triterpenes have got much resemblance with the spectra of the steroids. However in C-3 ketones of the series of steroids, the C-2 and C-4 methylene groups absorb near 1420 cm^{-1} while in the corresponding 3-oxo triterpenes, the C-2, methylene group absorbs near 1430 cm^{-1}, a C-11 methylene in 12-oxo steroids absorbs at 1434 cm^{-1}, whereas the same group in 12-oxo-triterpenes absorbs close to 1420 cm^{-1}. Cole and coworkers[52] have summarised the positions of carbonyl bands, ethylenic double bands[53] and the equatorial or axial nature of the hydroxyl groups, in triterpenic compounds[54] in the ir region.

As a result of infrared spectroscopic studies it might be possible to make a distinction between tertiary equitorial (3613 cm^{-1}) and axial (3617 cm^{-1}) hydroxyl groups, on this basis the band at 3629 cm^{-1} (CCl_4) in methyl melaleucate[55] (73) has been assigned as equitorial secondary, while its 3-α epimer, obtained by oxidations of the ketone (73) and subsequent reduction gives a band at 3636 cm^{-1} due to axial secondary nature of OH group.



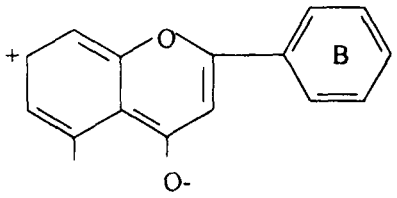
(73)

2. Ultra-Violet Spectroscopy:

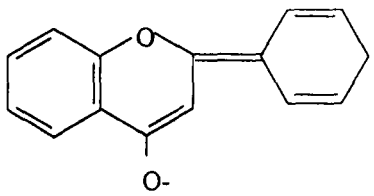
UV spectroscopy has become a major technique for the structure analysis of flavonoids for two main reasons. The first is that only a small quantity of pure material is required. The second reason is that the structural information obtained

about flavonoid from **uv** is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid nucleus. The addition of these reagents separately to an alcoholic solution of the flavonoid induce structurally significant shifts in the **uv** spectrum. The commonly used shifts reagents[56] are sodium methoxide (NaOMe), sodium acetate (NaOAc), sodium acetate / boric acid (NaOAc / H_3BO_3), aluminium chloride ($AlCl_3$) and aluminium chloride/hydro-choloric acid ($AlCl_3$/HCl).

The **uv** spectra of most flavonoids consist of two major absorption maxima, one of which occurs in the range 240-285 nm (band II) associated with ring-A benzoyl system (74) and second at a higher wave length (band I), occurs in the range of 320-380 nm associated with ring-B cinnamoyl absorption (75).[57]



(74)



(75)

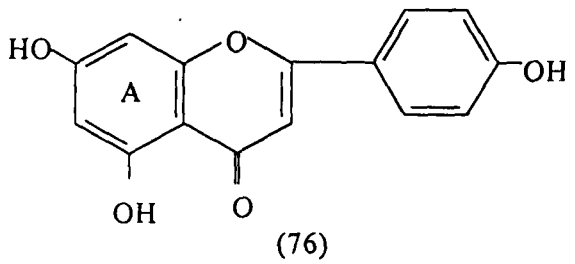
Substitution in ring-B specially at 4' stabilizes the cinnamoyl chromophore resulting in a red shift of band I whereas, substitution in ring-A has a similar effect on the position of band II. The presence of a free hydroxyl group at C-5 and C-3 positions is established by measuring the spectra in the presence of $AlCl_3$[56]. Compounds having a free 5-hydroxyl group absorb at higher wave length and methylation of this hydroxyl group brings about a blue shift of 1-15 nm of both bands. The hydroxyl groups at C-7 and 4' positions are more acidic than others and their occurrence is established by red shifts of band I & band II on the addition of fused sodium acetate.[56] The presence of hydroxyl group at 4' position is also confirmed by a large red shift in band I without a decrease in intensity on the addition of sodium methoxide[56]. The presence of ortho-dihydroxy groups in ring-A and ring-B is identified by a red shift in band I in the presence of $AlCl_3$ / HCl and sodium acetate/boric acid respectively.

In flavanones and isoflavones, due to the absence of cinnamoyl chromophore the high wave length band is either totally absent or present only as an inflection. Thus it is difficult to distinguish between flavanones and isolfavones with the help of **uv** spectrum alone. The **ultra-violet** spectra of biflavonoids are very similar to those of monoflavonoids with the only difference that the molecular extinction coefficient (ε) of the biflavone is approximately double as compared to the corresponding monoflavonoids. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of biflavonoids.

3. Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy:

Since flavonoid compounds contain, in general, very few protons, nuclear magnetic resonance spectroscopy is a useful tool in the structural elucidation of this class of compounds. By the use of **^{1}H-nmr** studies of silyl derivatives,[58] double irradiation techniques[59a], solvent induced shift studies[59b-61] and lanthanide induced shift studies[62] (LIS), one can come to the structure of flavonoids without tedious and time consuming chemical degradation and synthesis.

The valuable contributions in this field have been made by Batterham & Highet[63], Mabry[64] Massicot[65a,b], Clark-Lewis[66], Kawano[62,67], Pelter and Rahman.[68-70] **^{1}H-NMR** spectroscopy is highly helpful in determining the substitution pattern of flavonoids. The most commonly occurring hydroxylation pattern in natural flavonoids is 4', 5, 7-trihydroxy system (76).



(76)

^{1}H-NMR signals in trimethyl silylated Flavonoids[56] normally occur between 0 and 9 ppm. The chemical shifts of the protons of ring-A & B prove to be independent of each other but are affected by the nature of ring-C.

The signals arising from ring-A protons in most flavonoids occur upfield from those of ring-B protons, and are readily recognized. Different types of substitution in ring-A among the flavonoids and their effects on the proton signals can be discussed as follows.

(i) **H-5, H-6 and H-8 signals in 7-oxygenated flavnoids:**

The additional C-5 proton in these compounds is strongly deshielded by the 4-keto group and its signal appears at a very low field (δ 8.0). It appears as a doublet (J=9 Hz) due to ortho coupling with H-6. The signals for H-6, a d,d (q, J=9 Hz and 2.5 Hz) and for H-8, a doublet (d, J=2.5 Hz) occur at lower field than in the 5, 7-dihydroxy flavonoids and may even reverse their positions relative to one another.

(ii) **H-6 and H-8 signal in 5,7-dioxygenated flavonoids:**

The two ring-A protons, H-6 and H-8 give rise to two doublets (J=2.5 Hz) in the range δ 5.7-6.9 in flavones, flavonols, and isoflavones. The H-8 doublet occurs consistently downfield than the signal for H-6. The doublets for H-8 and H-6 are also clearly distinguished from each other by their widely different paramagnetic induced shifts. Depending upon the nature of the substituents, the chemical shifts may vary accordingly. For instance when a sugar is attached to the oxygen at C-7, the signal for both H-6 and H-8 are shifted downfield.

(iii) **H-6/H-8 signal in 5, 7, 8/5,6,7-trisubstituted flavonoids:**

^{1}H-NMR provides the requisite information for differentiating 6 or 8 substituted isomers of 5,7,8 / 5,6,7-trisubstituted flavonoids with a high degree of surety. Horowitz & Gentili[71] were able to fix up the structure for the two isomers of vitexin, viz. vitexin and isovitexin. The H-6 proton signal appears at about δ 0.2-0.3 upfield than H-8 signal.

All ring-B protons appear around δ 6.7-7.9, a region separate from the usual ring-A protons. The signal for the aromatic protons of an unsubstituted ring-B in a flavone appears as a broad peak centered at about δ 7.45. The presence of ring-C double bond causes a shift of 2', 6'-protons and the spectrum shows two broad peaks, one centered at ≈ δ 8.00 (2',6') and the other at ≈ δ 7.6 (3',4',5')[62]. The presence of substitution in one or more positions causes a distinct change.

(i) **H-2',6' & H-3',5' signals in 4' oxygenated flavonoids:**

With the introduction of 4'hydroxyl group, the ring-B protons appear as a typical four peaks pattern of two doublets called A_2B_2 pattern (J=8 Hz, each). The H-3' and H-5' doublet always occurs upfield as compared to the H-2',6' doublet. This is attributed to shielding effect of the oxygen substituent and to the deshielding influence of ring-C functions on H-2' and H-6'. The position of H-2' and H-6' signal also depends to some extent on the oxidation level of ring-C.

(ii) **H-2', H-5' and H-6' signals in 3',4' -dioxygenated flavonoids:**

The ^{1}**H-nmr** spectrum of 3',4'-dioxygenated flavonoids gives the normal ABX pattern. The H-5' proton in flavones and flavonols in such system appears as a doublet centered between δ 6.7 and 7.1 (J=8 Hz) and the H-2' and H-6' signals which often overlap, usually between δ 7.2 and 7.9.

(iii) **H-2' and H-6'signals in 3',4',5'-trioxygenated flavonoids:**

In 3',4',5'-trihydroxylated flavonoids H-2' and H-6' are equivalent and appear as a two protons singlet in the range δ 6.5-7.5. Methylation shifts the signal to downfield by about 1 ppm when the compound is analysed in DMSO-d_6.

(iv) **H-2 and H-3 signals in flavanones and flavanonols:**

The spectra of flavanones (saturated heterocyclic ring) contain typcial ABX pattern multiplets arising from the C-2 proton and the two C-3 protons. The C-2 proton is splitted by the C-3 protons into a doublet of doublet (J_{CIS}=5 Hz, J_{trans}=11 Hz.) and occurs near δ 5.2, the precise position depending on the substitution of ring-B. The two C-3 protons occur as two quartets ($J_{H\text{-}3a,\ H\text{-}3b}$=17 Hz) at δ 3.0. However, they often occur as two doublets, since two signals of each quartet are of low intensity.

The C-2 proton in dihydroflavonols appears near δ 4.9 as a doublet (J=11 Hz) coupled to the C-3 proton which appears at about δ 4.2 as doublet.[72]

Hydroxy protons:

The position of hydroxyl groups in flavonoids can not be detected by **^{1}H-nmr** spectra of their trimethylsilylated derivatives and thus can't be used for their detection. The **^{1}H-nmr** spectra of parent compound in DMSO-d_6, however, can give good information for the detection of phenolic hydroxyl protons. The hydroxyl protons of 3,5,7-trihydroxylfavone give three signals at δ 12.40 (5-OH), δ 10.93 (7-OH) and δ 9.70 (3-OH).[56]

Sugar protons:

The sugar protons in the flavone glycosides are denoted as C-1", C-2" protons and so on, while as the protons of the terminal sugar in disaccharides are designated as C-1"', C-2"' protons and so on. In the **^{1}H-nmr** spectra of TMS derivatives of the glycosides, the non anomeric protons resonate between δ 2.9-4.3, while the anomeric protons resonate between δ 4.3-5.8. The axial anomeric protons are observed between δ 4.3-5.0 and the equatorial anomeric protons between δ 4.7-5.8.

The chemical shift of the C-1" protons of the sugar directly attached to the flavonoids hydroxyl group depends both on the nature of the flavonoid and on the position and stereochemistry of the attachment. For instance, in flavone glycosides with sugar on either C-5, C-7 or C-4', the C-1" proton signal appears near δ 5.0, while in flavonols 3-0-glycosides the C-1" proton signal appears much more downfield i.e. at about δ 5.8. The coupling constant of C-1" proton with C-2" proton in β-linked glycosides is about 7 Hz[56], due to diaxial coupling. In the naturally occurring α-linked rhamnosides, the diequitorial coupling between H-1" and H-2" gives rise to a coupling constant of only 2 Hz[73]. The rhamnose C-methyl appears as a doublet (J=6.5 Hz) or a multiplet in the region δ 0.8-1.2.

In flavonoid diglycosides, the C-1" proton of the terminal sugar (H-1"') being relatively remote from the flavonoid nucleus, resonates upfield from H-1". The extent, however, can vary depending upon the position of attachment of terminal sugar.[74] Methylated[57] and acetylated[57, 74-75] derivatives have also been used for disaccharide linkage determinations.

Acetoxyl and Methoxyl protons:

In the **^{1}H-nmr** spectra of acetylated flavonoids ($CDCl_3$), the position of methyl signals of acetyl groups can also give useful information about the position of acetyl group by which the position of the hydroxyl groups can be confirmed. The methyl signals of 4' and 7-O-acetyl groups appear in the range of δ 2.30-2.35. While the methyl signal of a 5-0-acetyl group appears at about δ 2.45. The aliphatic acetoxyl signals of sugars generally appear in the range of δ 1.65-2.10. The position of the aliphatic acetoxyl groups of sugars also help in the location of sugar moiety in C-glycoxyl flavonoids.[57] Within the aliphatic acetoxyl group signals, the 2"-O-acetyl signal appears at δ 1.70-1.75 in 8-C-glycosylflavonoids and δ 1.80-1.83 in 6-C-glycosylflavonoids and 6"-0-acetyl signal in 8-C-glycoxyl flavonoids appears at δ 1.90-1.95 while in 6-C-glycosylflavonoids it appears between δ 1.98-2.04. Methoxyl protons signals[51, 65a], with few exceptions appear in the range of δ 3.5-4.1.

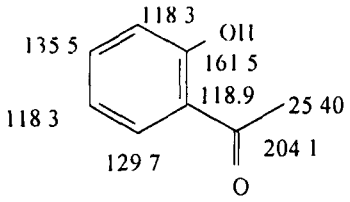
(4) ^{13}C-NMR Spectroscopy:

^{13}C-NMR spectroscopy has been used in natural product chemistry in variety of ways at various stages of the structure determination. **^{13}C-NMR** spectral data furnish key informations such as the number of carbon atoms and establish if they are primary, secondary, tertiary, aromatic, olefinic or part of functional groups.

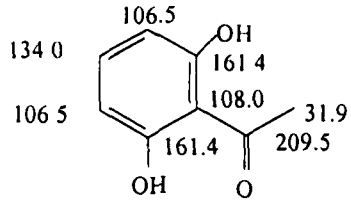
The **^{13}C-nmr** spectra of flavonoids and their glycosides[76,85] are of some interest in the context of compounds isolated during the course of this work. The spectra can be analysed by reference to those of simple compounds such as acetophenones[76-77] and cinnamic acids[77] which possess structural features characteristic of flavonoids.

It is worthwhile to see how introduction of oxygen at various positions of these, effects the chemical shifts. In hydroxy acetophenone (77) the nuclear carbon linked directly to oxygen of hydroxyl group gives rise to a singlet at δ 161.5 and the two adjacent carbons give two singlets at δ 118.0. The carbon para to the carbonyl is the most deshielded and its singlet appears at δ 135.5. In 2,6-dihydroxy acetophenone (78) the carbon bonded directly to oxygen give rise to singlet at δ 161.4 and the two

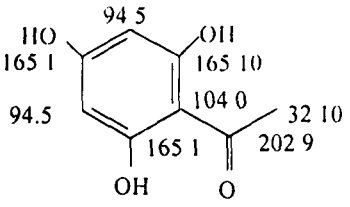
adjacent carbons produce singlet at δ 106.5. The meta carbon which is para to the acetyl group is deshielded and its singlet appears at δ 134.0.



(77)

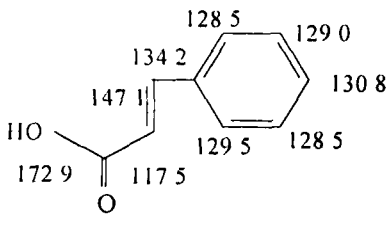


(78)

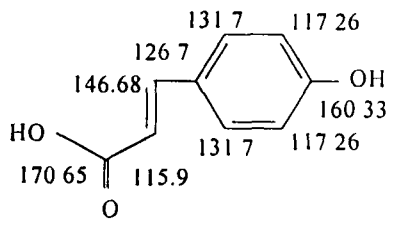


(79)

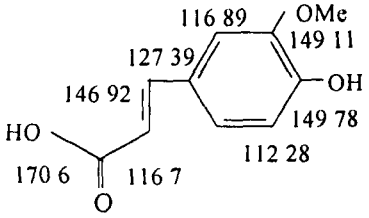
Thus, chemical shifts correlate to those for protons on these carbons, the protons ortho and para to hydroxyl being shielded more than the one at meta positions and protons para and ortho to carbonyl being the ones most exposed to the deshielding influence of the carbonyl group. In 2,4,6-trihydroxy acetophenone (79) the oxygenated nuclear carbons show singlet at 165.10 pm while in dihydroxy acetophenone (78) it is δ 161.4. This slight deshielding of δ 3.70 can be attributed to the hydroxyl group at meta position. The unsubstituted carbons 3 & 5 are shielded due to enhanced mesomeric effect and their signals appear at δ 94.5. These effects can be assumed to be general and are relied upon in making assignments in flavonoid spectra. The other structural unit of flavonoids is akin to cinnamic acid and the ^{13}C-**nmr** chemical shifts of cinnamic acid derivatives are, therefore, of interest. The chemical shifts of the parent cninamic acid and its mono-di and trisubstituted derivatives are indicated in the structure (80, 81, 82, 83).



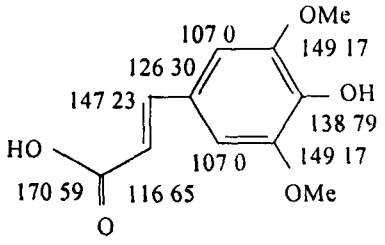
(80)



(81)



(82)



(83)

The 3,4-type of substitution is the one most commonly encountered in flavones and chemical shifts of carbon 3 and 4 of 3-methoxy 4-hydroxy cinnamic acid (82) δ 149 11 and δ 149 78 respectively are substantially different from those of carbons under oxygen in acetophenone. This makes it possible to distinguish between oxygenated ring-A and B carbons of flavones. The carbons ortho and meta to phenolic hydroxyls are shielded, compared to unsubstituted benzene and appear at δ 112 28 and δ 116 89, the cinnamic acid double bond causing a further shift of C-2 resonance Carbon-1 adjacent to the olefinic double bond of cinnamic acid is almost at the same value as in substituted benzene but different in unsubstituted benzene The α-carbon appears at δ 117 5 and the β-carbon at δ 147.1. In trisubstituted benzene (83), the carbons attached to oxygen are further shielded and in 3,5-dimethoxy 4-hydroxy cinnamic acid appear at δ 149.17, δ 138.79 respectively. The carbon bearing hydroxyl is shielded to a greater extent because of resonance contribution from the flanking methoxyl groups The same type of resonance effect is responsible for the shielding of 2 and 6 carbons

The chemical shifts of flavones, substituted flavones and isoflavones are reproduced[76] in the following table

Chemical shift (in δ dowinfield from T.M.S.)

Carbon Number	Flavone	7-methoxy flavone	5-hydroxy flavone	5,7,3'4'-tetrahydroxy Flavones	7-methoxy isoflavone
2.	163.2	162.6	164.07	165.07	152.4
3.	107.6	107.2	105.61	103.94	125.1
4.	178.4	177.4	182.90	182.63	175.3
5.	125.2	126.7	155.85	158.24	127.6
6.	125.2	114.1	107.22	94.90	114.6
7.	133.7	163.7	135.61	164.34	163.8
8.	118.1	100.2	110.83	99.91	100.0
9.	156.3	157.7	159.82	161.56	157.7
10.	124.0	117.6	110.13	104.2	118.3
1'	131.8	131.6	130.54	123.06	127.9
2'	126.3	125.8	126.39	114.38	128.2
3'	129.0	128.7	128.91	145.95	128.8
4'	131.6	131.1	131.97	149.84	131.8
5'	129.0	128.7	128.91	117.05	128.8
6'	126.3	125.8	126.39	120.14	127.9

(5) Mass Sepctrometry

The introduction of inlet system suitable for volatilization of high molecular weight (M^+, 300-1200) organic materials has greatly increased the utility of **mass** spectrometry. Generally the fragmentation is related to the structure of the intact molecule. Electron impact **mass** spectrometry of both flavonoid aglycones and glycosides serves as a valuable aid in determining their structures, especially when only very small quantities (i.e. less than 1 mg) of the compounds are available. It has been applied successfully to all classes of flavonoid aglycones and also a number of different types of glycosides.[79-84] The flavonoid aglycones and glycosides have been subjected to **GC-MS** spectrometry in the form of their permethyl ethers, perdeuteriomethyl ehters[85-86] and trimethylsilyl ethers[87-88].

Flavones:

Most flavonoids yield intense peak for the molecular ion ($M^{+\cdot}$) and indeed this is often the base peak. In addition to the molecular ion, flavonoids usually afford major peaks for [M-H] and, when methoxylated, [M-CH_3]. Perhaps the most useful fragmentation in terms of flavonoid identification are those which involve the cleavage of intact A and B-ring fragments. Kingston[89] had discussed in detail the mass spectra of large number of flavones, flavonols, flavanones and their ether derivatives **(Scheme I, II & III).** The fragmentation pattern of monoflavones has been summarised as follows :

a) Flavones with fewer than four hydroxyl groups do not readily fragment, a consequence of the stability of their molecular ion.

b) Flavones with fewer than four hydroxyl groups tend to undergo decomposition predominantly by way of the retro-Diels-Alder (RDA) process.[83-84] This and other common fragmentation processes are shown in **(Scheme-I)** using apigenin (84)[83] as a typical example

c) An [M-1] ion is often found in the mass spectra of flavones, its origin is however, obscure.

d) The presence of ion m/z 137 **(Scheme-II),** frequently more intense when a 3-hydroxy group is present, is attributed to the alternative mode of retro-Diels-Alder fragmentation.

e) Doubly charged ions are frequently present.

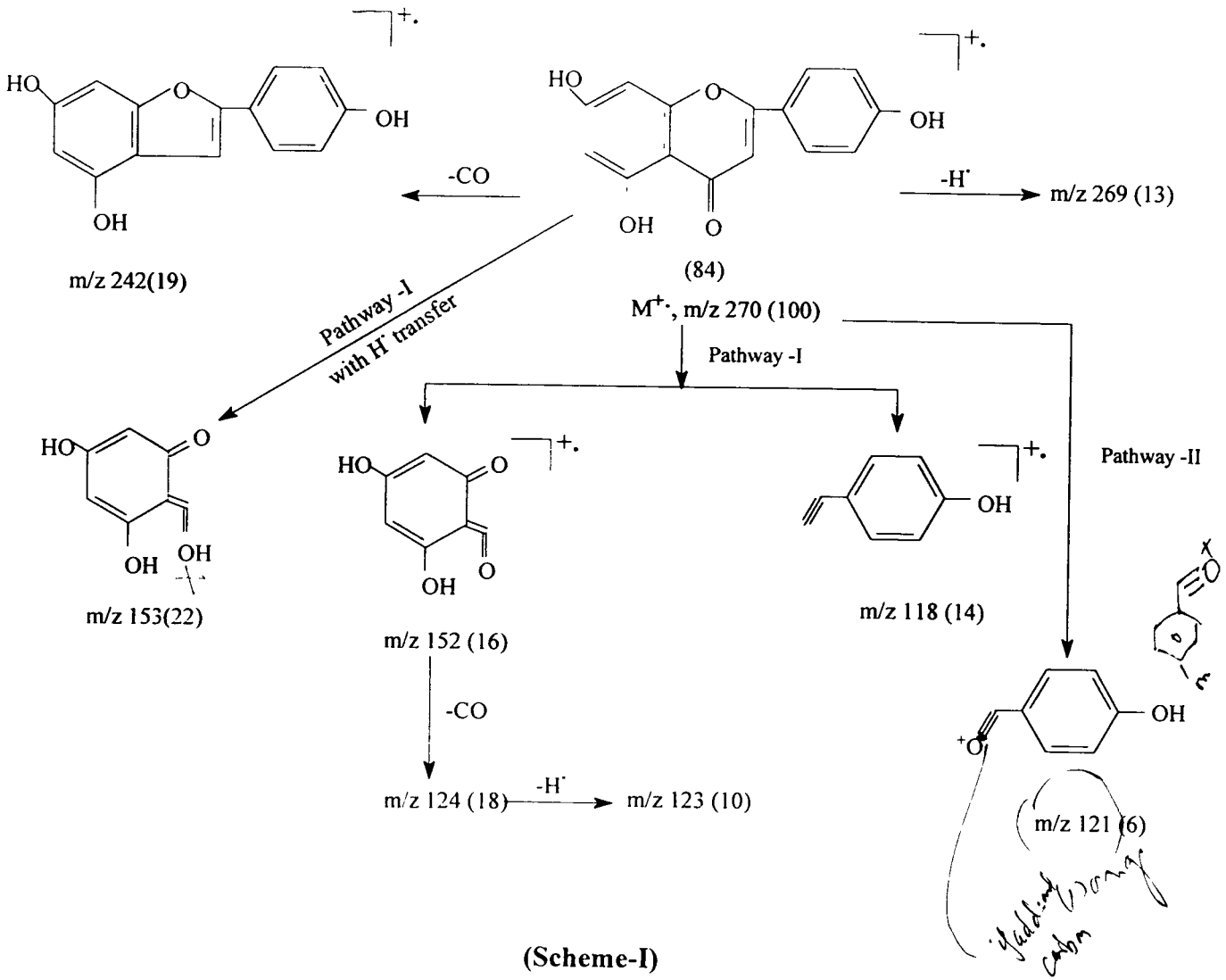
f) When heavily substituted with hydroxyls and methoxyls, the flavones tend to fragment in a less predictable manner, retro-Diels-Alder process becomes insignificant and the spectrum is dominated by the molecular ion and ions at M-15, M-28 and M-43.[85,87]

Flavonoid O-glycosides:

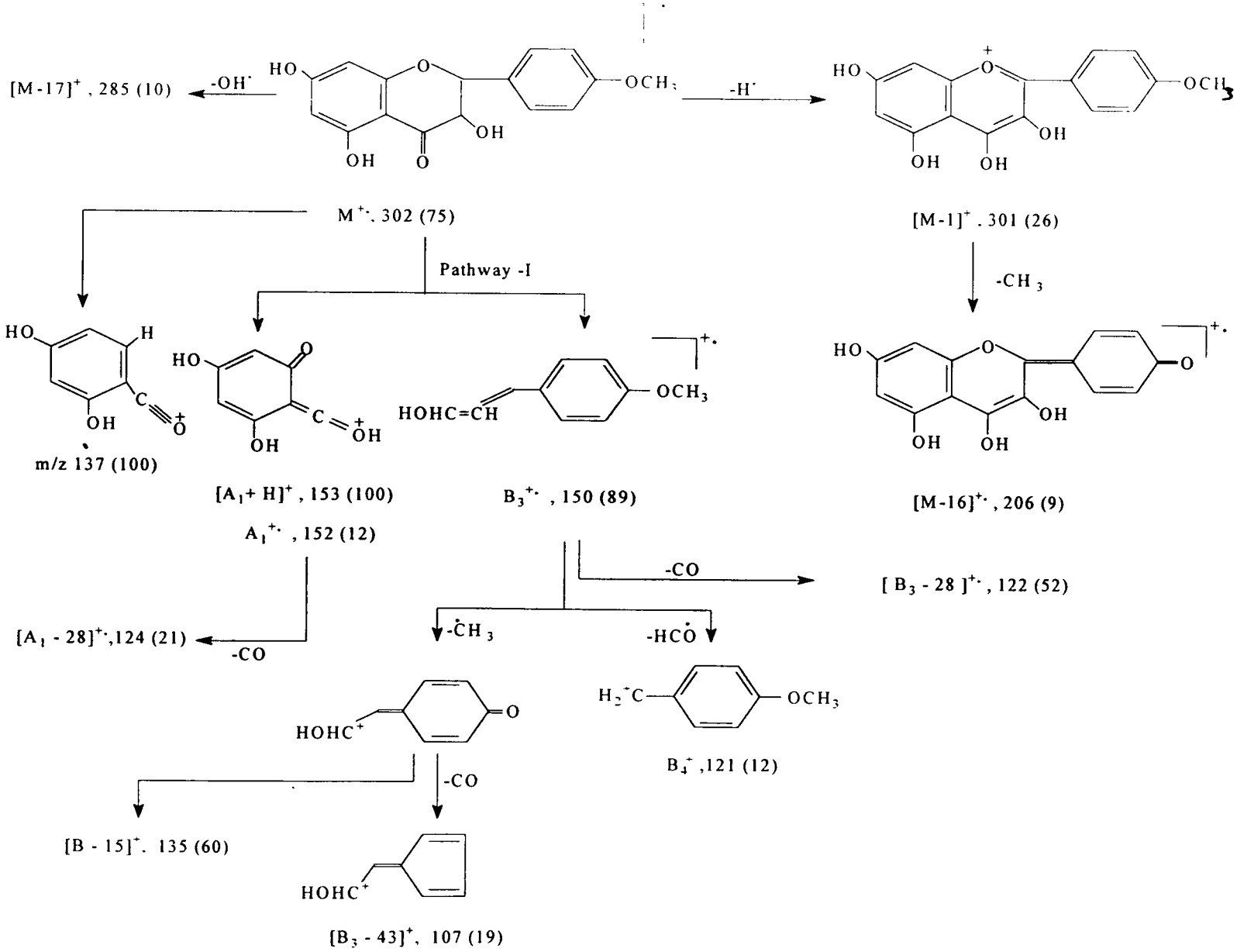
The position of a sugar residue in a flavonoid aglycone can be easily recognized from the mass spectrum of permethylated glycosides.[88] The sugar attached

to the position 5 and 3 splits more readily than that at position 7 and as a result the molecular ion peak is of very low intensity or totally absent.

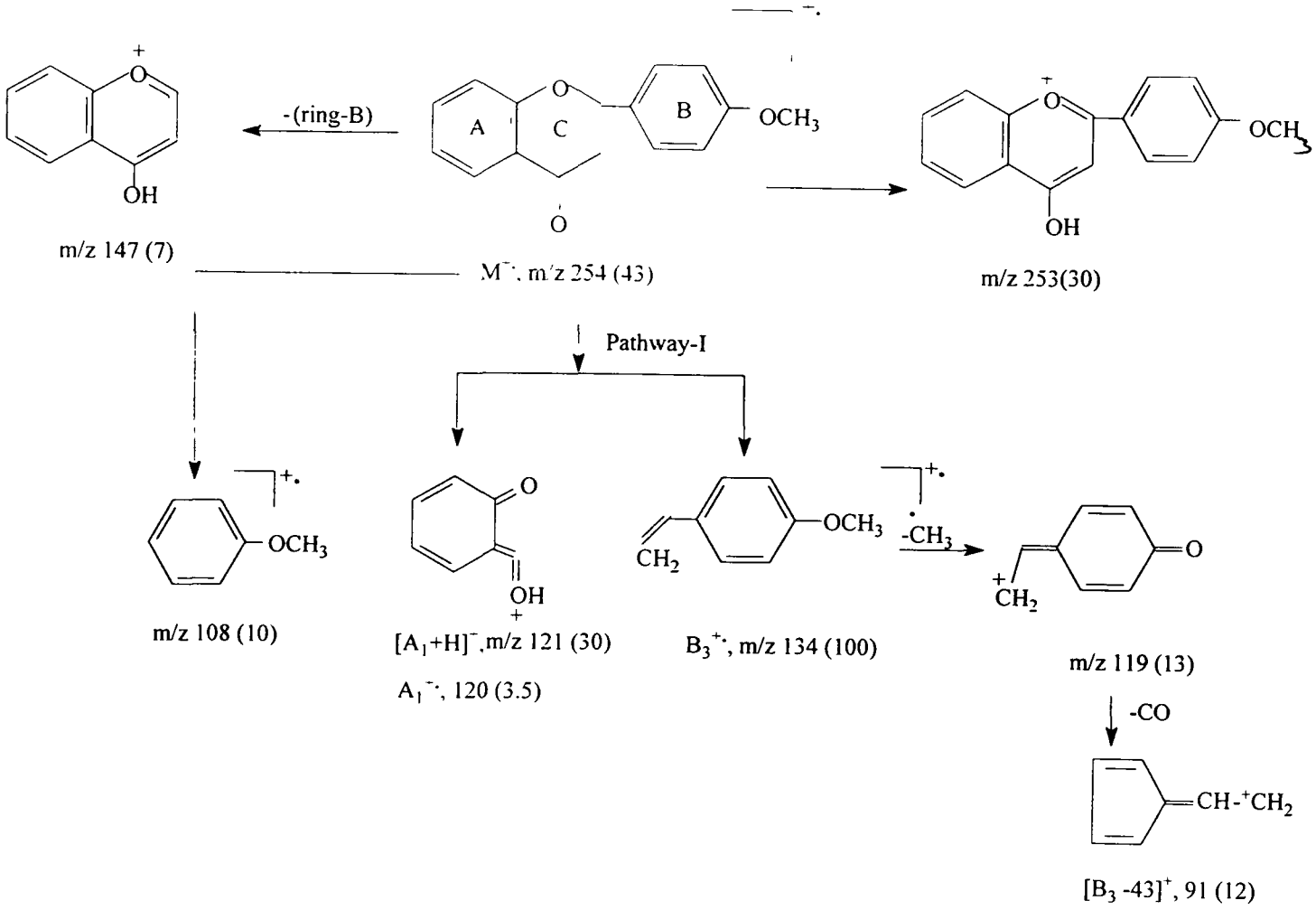
On the other hand 7-O-glycosides usually show an intense molecular ion peak amounting to 50% or higher. The 4'-glycosides represent an intermediate case, having small but distinct molecular ion peak.


HO
O
OH
OH
-CO
m/z 242(19)
HO
O
OH
OH
O
-H·
m/z 269 (13)
(84)
M+·, m/z 270 (100)
Pathway -I with H· transfer
Pathway -I
Pathway -II
HO
O
OH
OH
m/z 153(22)
HO
O
OH
O
m/z 152 (16)
-CO
m/z 124 (18)
-H·
m/z 123 (10)
OH
m/z 118 (14)
OH
m/z 121 (6)

(Scheme-I)


[M-17]+ , 285 (10)
-OH·
HO
O
OCH3
OH
OH
O
-H·
[M-1]+ . 301 (26)
M+·, 302 (75)
Pathway -I
-CH3
m/z 137 (100)
[A1+ H]+ , 153 (100)
B3+· , 150 (89)
[M-16]+· , 206 (9)
A1+· , 152 (12)
-CO
[B3 - 28]+· , 122 (52)
[A1 - 28]+·,124 (21)
-CO
-·CH3
-HCO·
HOHC+
H2+C
B4+ ,121 (12)
-CO
[B - 15]+. 135 (60)
[B3 - 43]+, 107 (19)

(Scheme-II)


-(ring-B)
A
C
B
OCH3
OH
m/z 147 (7)
M+·, m/z 254 (43)
m/z 253(30)
Pathway-I
m/z 108 (10)
[A1+H]+,m/z 121 (30)
A1+·, 120 (3.5)
B3+·, m/z 134 (100)
-CH3
m/z 119 (13)
-CO
CH-+CH2
[B3 -43]+, 91 (12)

(Scheme-III)

REFERENCE

Some refrences have titles while others don't? Be consistent. Also don't have initials.

1. J.B. Harbone, T.J. Mabry and H. Mabry (Ed.), **'The Flavaonoids'** Champman and Hall, London, p. 1035-1042 (1975).
2. Y. Tsuchiya, M. Shimizu, Y Hiyama, K. Itoh , Y. Hasahimoto, M. Nakayama, T. Horie, N. Morita, **Chem. Pharm. Bull., 33** (9), 3881-86 (1985).
3. M. Gabopr, **'The anti-inflammatory action of flavonoids,** Akademiaj Kiado, Budapast (1972).
4. J.F. Marton, Quart, **J. Crude Drug Research, 12**, 1829 (1972).
5. J.P.S. Sarin, S. Singh, H.S. Garg, N.M. Khanna and M.M. Dhar, **Phytochemistry, 15** 232 (1976).
6. T. Kinoshita, U. Sankawa, T. Takuma, K. Asahl, N. Takahashi, **Chem Parm Bull., 33** (9), 4109-12 (1986).
7. M.V. Quarenghida Riea, P. Seeligmann, **Acta. Farm Bouareuso, 4,** 33-5 (1985), Chem. Abs. 101945, Vol. 104 (1986).
8. K. Yasukawa, M. Takipo, M. Taki and Shigeki Nakagawa, **Chem. Pharm, Bull., 37(4),** 1071-1073 (1989).
9. **Glimpses in Plant Research**, Vol. XI, 377-389 Medicinal Plants: New Vistas of Research (Part-2) (1993).
10. H. Kumamoto, Y. Matsubara, Y. Lizuka, K. Okamato and K. Yoko, **Nippon Nogeikagaken Kaishi, 59**, 677 (1985).
11. `Kitaoka, M. Kadokawa, H., Sugano, M., Ichikawa, K., Taki, M., Takaishi, S., Iijima, Y., Tsutsumi, S., Boriboon, M., Akiyama, T., Prenylflavonoids: A new class of non-steroidal phytoestrogen (Part I). Isolation of 8-iospentenyl-naringenin and an initial study on its structure-activity relationship. **Planta Medica 64**, 511-515 (1998).
12. Middleton Jr. E., Kandaswami, C. The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In: Harborne, J.B. (Ed.). **The Flavonoids: Adances in Research Since 1986.** Champman & Hall London, pp. 619-652 (1994).
13. Mata, R., Rojas, A., Acevedo, L., Estrada, S., Calzada, F., Rojas, I., Bye, R., Linares, E. Smooth muscle relaxing flavonoids and terpenoids from *Canyza filaginoides*. **Planta Medica 63,** 31-35 (1997).

14. Middleton Jr, E., Kandaswami, C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne, J.B., (Ed.). T**The Flavonoids: Advances in Research Since 1986**. Champman & Hall, London, pp. 619-652 (1994).

15. Chen, T., Li, J., Cao, J., Xu, Q., Komatsu, K., Namba, T.. A new flavonone isolated from *Rhizoma smilacis glabrae* and the structural requirements of its derivatives for preventing immunological hepatocyte damage. **Planta Medica 65,** 56-59 (1999).

16. Sekine, T., Inagaki, M.,Ikegami, F., Fujii, Y., Ruangrungsi, N. Six diprenylisoflavones, derrisisoflavones A.F. from *Derris scandens*, **Phytochemistry 52,** 87-94 (1999).

17. Kazutaka Nishikawa, Hiroko Furukawa, Toshihiro Fujioka, Hiroki Fujii, Kunihide Mihashi, Koichiro Shimomura, Kanji Ishimaru., **Phytochemistry, 52**, 885 (1999).

18. Davyson de L. Moreira, Elsie F. Guimaraes, Maria Auxiliadora C. Kaplan, **Phytochemistry, 55,** 783 (2000).

19. Tsutomu Hatano, Seiki Mizuta, Hideyuki Ito, Takashi Yoshida, **Phytochemistry, 52,** 1379 (1999).

20. Salwa F. Farag, Ennam Y. Backheet, Nasr A. El-Emary, Masatake Niwa, **Phytochemistry, 50,** 1407 (1999).

21. A.G. Damu b. Jayaprakasam, D. Gunasekar, A. Blond, B. Bodo, **Phytochemistry, 52,** 147 (1999).

22. Jose I. de Souza Alberto C. Arruda, Gaspar D. Munoz, Mara, S.P. Arruda, Adolfo H. Muller, **Phytochemistry, 52,** 1705 (1999)

23. Petra Mann, Britta Tofern, Macki Raloga, Eckart Eich., **Phytochemistry, 50,** 267 (1999).

24. Ying Huang, Tess De Bruyne, Sandra Apers, Yuliang Ma, Magda Claeys, Luc Pieters, **Phytochemistry, 52,** 1701 (1999).

25. Eliane O. Ferreira, Diones Dias, **Phytochemistry, 53,** 145 (2000).

26. Md. Tofazzal Islm, Satoshi Tahara, **Phytochemistry, 54,** 901 (2000).

27. Fabio D.P. de Andrade, Lourdes C. dos Santos, Anne L. Dokkedal, Wagner vilegas, **Phytochemistry, 51,** 411 (1999).

28. Mona-Antonia Beck, Hanns Haberlein, **Phytochemistry, 50,** 329 (1999).

29. Ahmed A. Gohar, Galal T., Maatooq, Masatake Niwa. **Phytochemistry, 53,** 299 (2000).

30. Dagoberto Alavez-Solano, Ricardo Reyes-Chilpa, Manuel Jimenez-Estrada, Federico Gomez-Gariboy, Isabel Chavez-Uribe Mario Sousa-Sanchez, **Phytochemistry, 55,** 953 (2000).

31. Eckhard Wollenweber, Rudiger Wehde, Marion Dorr, **Phytochemistry, 55,** 965 (2000).

32. F.N. Ngounou, A.L. Meli, D. Lontsi, b.L. Sondengam, Attaur-Rahman, M. Iqbal choudhary, Shahid Malik, Farzana Akhtar, **Phytochemistry, 54,** 107 (2000).

33. Salwa F. Farag, Enaam Y. Backheet, Nasr A. El-Emary, Masatake Niwa, **Phytochemistry, 50,** 1407 (1999).

34. Joao B.F. Tostes, Antonio J.R. Silva, Jose P. Parente, **Phytochemistry, 50,** 1087 (1999).

35. Alfonse Silayo, Bonaventure T. Ngadjui, Berhanu M. Abegaz, **Phytochemistry, 52,** 947 (1999).

36. Toshikazu Sekine, Miyuki Inagaki, Fumio Ikegami, Yukchi Fujii, Nijsiri Ruangrungsi, **Phytochemistry, 52,** 87 (1999).

37. Yoshiaki Shirataki, Satoko Matsuoka, Manki Komatsu, Masyoshi, Ohyama, **Phytochemistry, 50,** 695 (1999).

38. Fabiana N. Fonseca, Ari J.S. Ferreira, Patricia Sartorelli, Norberto P. Lopes, Fny I.S. Floh, Walter Handro, Massuo J. Kato, **Phytochemistry, 55,** 575 (2000).

39. Eike Brmkmeier, Hans Geiger, Hans Dietmar Zinsmeister, **Phytochemistry, 52,** 297 (1999).

40. B. Jayaprakasam, A. G. Damu, D. Gunaseker, A. Blond, A.B. Bodo, **Phytochemistry, 53,** 515 (2000).

41. Kan Chantra Promma, Yanisa Rat-A-Pa, Chatchanok Karalai, Vitchu Lojanapiwatana, Vatcharee Seechamnan-turakit, **Phytochemistry, 53,** 511 (2000).

42. Ishmael B. Masesane, Samuel, O. Yeboah, Jurgen Liebscher, Clemens Mugge, Berhanu M. Abegaz, **Phytochemistry, 53,** 1005 (2000).

43. Kan Chantra Promma, Yanisa Rat-A-Pa, Chatchanok Karalai, Vitchu Lojanapiwatana, Vatcharee Seechmnan-turakit, **Phytochemistry, 53,** 511 (2000).

44. Cesor C. andrei, Dalva T. Ferreira, Milton Faccione, Luiz Alberto B. de Moraes, Mario G. de Carvalho, Raimundo Braz Filho, **Phytochemistry, 55,** 799 (2000).

45 Daniela M. Tomazela, Monica, T. Pupo, Edna A. P. Passador, M. Fatima das G.F da Silva, Paulo, C. Vieira, Joao B. Fernandes, Edson Rodrigues F.O , Glaucius Oliva, Jose, R. Pirani, **Phytochemistry, 55,** 643 (2000).

46 H.M.T.B. Herath, P.S. Athaukoralage, Joanne F. Jamie, **Phytochemistry, 54,** 823 (2000)

47 Salvatore de Rosa, Carmine Iodice, Maya Mitova, Nedjalka, Handjieva, Simeon Popov, Mincho Anchev, **Phytochemistry, 54,** 751 (2000).

48 N Kakazawa, **Chem. Pharm. Bull**. Tokyao, **16**, 2503 (1968)

49 J. Shinoda, **J. Pharm. Soc. Japan**, **48**, 214 (1928).

50 S Beckmann, H Geiger and W. de Grootpteider, **Phytochemistry**, **10**, 2465, (1971)

51 J. H. Looker and W.W. Hanneman, **J. Org. Chem.**, **27**, 381 (1962).

52 A R.H Cole, Zeichmester's Progree in Chemistry of Organic Natural Products (Wein, spruiger Verlag, New York), XIII, **60** (1956).

53. A.R.H. Cole and W. Thorntop, **J. Chem. Soc**., 1332-38 (1957).

54 Miss I L Allseep, A.R.H., Cole, D.E. White and R.L.S. Willix, **J. Chem. Soc.,** 4268 (1956).

55 H.R. Arthur, A.R.H. Cole, K.J.L. Thiebery and D.E. White, **Chem. & Ind.,** 926, (1956).

56 T J Mabry, K.R. Markhem and M.B. Thomas, **'The Systematic Identification of Flavonoids**, springer-Verleg, New York, Heidelberg, (1970).

57. R.M. Horowitz and B. Gentilli, **Chem. and Ind.** (London), 625 (1966).

58. A.C. Waiss Jr., R.E. Ludin and D.J. Stera, **Tetrahedron letters, 10**, 513 (1964).

59. V.V.S. Murti, P.V. Raman and T.R. Shesadri (a) **Tetrahedron, 23**, 397 (1967); (b) **Tetrahedron Letters, 40**, 2995 (1964).

60. F.C. Chen, Y.M. Lin and J.C. Hung, **Phytochemistry, 14**, 818 (1975).

61. E. Rodriguiz, N.J. Carman and T.J. Mabry, **Phytochemistry, 11**, 409, (1972).

62. M. Okigawa, N. Kawano, W. Rahman and M.M. Dhar, **Tetrahedron Letters, 40**, 4125 (1972).

63. T.J. Batterham and R. J. Highet, **Aust.J. Chem., 17**, 428 (1964).

64. T.J. Mabry, J, Kagan and H. Rostel, Monograph, **'NMR analysis of flavonoids'**, Univ. of Texas, Publication No. 6418 (1964).

65. a) J. Massicot and J.P. Marthe, **Bull. Soc. Chem**. Fr., 2712 (1963).

b) J. Massicot and J.P. Marthe, **Bull. Soc. Chem**. Fre., 1962 (1962).

66. J.W. Clark-Lewis, L.M. Jackson and T.M. Spotswood, **Aust. J. Chem.** (a) **17**, 632 (1965); (b) **21**, 2059 (1968).

67. H.M. Miura, T. Kihara and N. Kawano, **Chem. Pharm. Bull**., Tokya, **17**, 150 (1969), **Tetrahedron Letters, 19**, 2339 (1968).

68. W. Rahman, Unpublished results.

69. M. Ilysa, J.N. Usmani, S.P. Bhatnagar, M. Ilyas, W. Rahman and A. Pelter, **Tetrahedron Letters, 53** 5515 (1968).

70. A. Pelter, R. Warren, J.N. Usmani, R.H. Rizvi, M. Ilyas, and W. Rahman, **Experintia** (a) **25**, 350 (1969), (b) **25**, 351 (1969).

71. R.M. Horowitz and B. Gentili, **Chemistry and Industry, 24**, 498 (1964).

72. K.R. Markham and T.J. Mabry, **Tetrahedron, 24,** 823 (1968).

73. T.J. Mabry, J. Kagan and H. Roser, **Phytochemistry, 4,** 487 (1965).

74. H. Rosler, T.J. Mabry, M.F. Cranmer and J. Kagan, **J. Org. Chem., 30** 4346 (1965).

75. V. Plouvier, **C.V. Acad. Sci., Paris Ser. D., 270**, 2710, (1970).

76. K.R. Markham, C. Sheppard, H. Geiger, **Phytochemistry, 26** (12), 335-3337 (1987).

77. S. Cathrine Benzuidenhout, Barend C.B. Bezuident, E. Vincent Brandt and Dannelferrira, **J. Chem. Soc. Perkin-Trans-1,** p. 1237-1241 (1998).

78. O.G.I. Kingston. **Tetrahedron, 27**,2691 (1971).

79. R. Madhav, **Tetrahedron Letters, 25**, 2017 (1969).

80. H.S. Garg and C.P. Witra, **Phytochemistry, 10**, 2787 (1971).

81. M. Konoshina, Y. Ikeshiro and S. Miyagawa, **Tetrahedron Letters, 48,** 4203 (1970).

82. R.G. Wilson and D.H. Williams, **J. Chem. Soc.** (C), 2477 (1968).

83. R.I. Reed and J.M. Wilson, **J. Chem. Soc**. (C), 5949 (1968).

84. A Pelter, P. Stainton and M. Barber, **J. Heterocyclic Chem. 2,** 262 (1965).

85. R. Mues, B.N. Tiemmermann, N. Ohno and T.J. Mabry, **Phytochemistry, 18** 1379 (1979).

86. M.L. Bouillant, A. Besset, J. Favre-Bonvin and J. Chopin, **Phytochemistry, 19**, 1755 (1980).

87. H. Schels, H.D. Zinsmeister and K. Pfleger, **Phytochemistry, 10**, 1019 (1977).

88. H.Schels, H.D. Zinsmeister and K. Pfleger, **Phytochemistry, 17** 523, (1978).

89. O.G.I. Kingston, **Tetrahedron, 27**, 2691 (19871).

90. J.H. Bowie and D.W. Cameron, **Aust. J. Chem., 19,** 1627 (1966).

91. O. Seligmann, H. Wagner, In 'Topic in **Flavonoid Chemsitry and Biochemsitry'** (L. Farkas. M. Gabov and F. Kallay eds.) Akademiai Kiadio, Budapest (1975).

CHAPTER-II

ACACIA TORTILIS

Results & ?

DISCUSSION

CHEMICAL CONSTITUENTS FROM THE LEAVE OF *ACACIA TORTILIS* (LEGUMINOSAE)

The genus *Acacia* comprising over 500 species, found in the warmer drier parts of the World, chiefly in Arab, Australia and Africa.[1] Species with pinnately ~~compound~~ leaves are found throughout the tropics, and the phyllodineous ones are natives of Australia. In India, there are about 22 indigenous species, distributed throughout the plains.

The indigenous species are thorny trees or shrubs and a few are also climbers. Some of *Acacia* are of considerable value for afforestation and reclamation of waste land. They are the good source for tannin, gum and timber.[1]

Acacia tortilis wild. **(Syn: A. Radiana Savi)** was collected from northern semi-desert region of Yaman, and it was found to be a very useful source of protein.[2] The cell wall constituents, acid detergent fibres and cellulose found in the leaves provide as nutrients for the animals as fodders.[3] ***Acacia tortilis*** is also used for smooth muscle relaxing.[4] Earlier investigations on this plant reported the isolation of apigenin, quercetin and isorhamnetin glycoside from leaves[5-6] and n-hexa-cosanol, betulin, α, β-amyrin, β-sitosterol from stem bark.[7]

Medicinal importance and scanty work on this plant accelerates our interest to carry out the comprehensive investigation of the plant ***Acacia tortilis.*** The present discussion deals with the isolation and characterization of the following compounds from the leaves of ***Acacia tortilis***.

1. **Lupan-3-ol, 12,20 diene** (new compound)
2. **Lupan-12, 20 diene 3 one** (new compound)
3. **Friedelin**
4. **β-amyrin**

5. **β-sitosterol**

6. **Apigenin**

7. **Luteolin**

8. **Quercetin**

9. **5,7-dihydroxy-4-p-methyl benzylisoflavone** (new compound)

10. **Vitexin**

11. **2',6'-dihydroxy chalcone-4'-O-glucoside** (new compound)

The dried and powdered leaves of ***Acacia tortilis*** (3 kg) procured from Yaman, were exhaustively extracted with light petroleum ether (60-80), benzene and finally with methanol. The petrol and benzene concentrates gave positive test for triterpenes.[8] On TLC examination, these concentrates showed number of spots in different solvent systems (petrol-benzene and petrol-ether) with the same R_f values. The above two concentrates were therefore mixed together. The combined concentrate was chromatographed over silica-gel column, using successively petrol (A), Petrol-benzene (9:1-B_1, 8:2-B_2, 7:3-B_3, 6:4-B_4, 1:1-B_5) and benzene (C) as eluting solvents. Appropriate fractions (ir, Spectra and TLC) were combined.

The fractions A and B_1 on concentration gave a yellowish green oil of fatty nature, and were not further examined.

The fractions B_2 and B_3 on TLC examination (silica-gel, petrol-benzene 1:1) showed two major spots with the same R_f values. The above two fractions were therefore mixed together and subjected to column chromatography over silica-gel followed by fractional crystallization, afforded two crystalline TLC homogenous ~~substances~~ compounds, marked as **At-1** and **At-2**.

The fractions B_4, B_5 and C were found to be having the same composition with varying concentrations of the compounds. The three fractions were combined

together. Repeated column chromatography over silica gel column using petrol-benzene mixture in different ratios gave three compounds containing some minor impurities. Repeated crystallization by an appropriate solvent, gave pure compounds labeled as **At-3, At-4** and **At-5.**

The methanol extract was concentrated by heating over a boiling water bath under reduced pressure, a brown gummy mass was obtained. It gave positive colour test for flavonoids[17]. The examination in TEF and BPF systems showed it to be mixture of several compounds. The brown gummy mass was purified by refluxing it with petroleum ether, benzene and chloroform. The semi-solid mass left behind was chromatographed over silica gel column. Fractional elution with benzene-ethylacetate (1:1) and ethylacetate yielded four compounds. They were purified by repeated crystallization and labeled as **At-6, At-7, At-8** and **At-9**. Further elution of the column with ethylacetate-methanol mixture gave two compounds labeled as **At-10** and **At-11**.

At-1

The compound **(At-1)** was crystallized from benzene-petrol as shining white needles m.p. 165-66^{0}C, $[\alpha]^{20}_D$ + 24.54 ($CHCl_3$). It gave a positive Leiberman-Burchard[14] and Nollers tests[8] and yellow colour with tetranitrom-ethane, indicating the presence of double bond. Elemental analysis agreed with the formula, $C_{30}H_{48}O$. The **infrared** spectrum **(Fig.-I)** of the compound **(At-1)** showed absorptions at υ^{KBr}_{max} 3360 and 1030 cm^{-1} (OH), 1630, 1445 cm^{-1} (C=C) and 1375 cm^{-1} (geminal dimethyl), 875 cm^{-1} (terminal methylene).

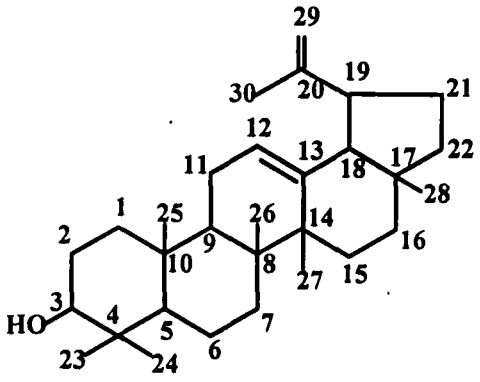
The **^{1}H-nmr** spectrum **(Fig.-II)** of the compound **(At-1)** revealed seven methyl groups at δ 0.82 (3H), 0.91 (6H) 0.93 (3H), 0.99 (6H) and 1.68 (3H). The CH_2 protons were evident by the signals in the range of δ 1.34-1.65. The terminal double bond (>C=CH_2) was indicated by the signals at δ 4.57 and 4.68 while the signal at δ 4.85 corresponded to characteristic olefinic proton. A multiplet at δ 3.20 was ascribed to C-3-OH. It was also supported by the **mass** spectrum **(Fig.-III, Scheme-I)** which showed the molecular ion peak ($M^{+\cdot}$)at m/z 424 (100%), with principal ions at m/z 409 ($M^{+\cdot}$-CH_3, 30%), 256 (9%), 207 (15%) and at m/z 188 (78%). The fragment ions at m/z 207,217 and 256 ~~representing~~ provided the presence of double bond at Δ^{12}-position.

The assigned structure was further confirmed by the **^{13}C-nmr** spectrum **(Fig.-IV)** in which the C-3-OH appeared at δ 78.88 and the olefinic carbons at 129.63, 142.68, 150.90, 109.25. The assignment of other carbons are shown in **(Table-1)**.[9]

Acetylation of **At-1** with acetic anhydride and pyridine gave a monoacetate m.p 152^{0}C its **^{1}H-nmr** spectrum **(Fig.-V)** showed five independent singlets of methyl groups at δ 0.87 (3H), 0.93 (6H), 0.96 (3H), 1.02 (6H) and 1.68 (3H). The

CH_2- protons appeared in the range of δ 1.36-1.59 and the terminal olefinic protons appeared at δ 4.56 and 4.68, while the signal at δ 4.85 indicated the Δ^{12} proton. A singlet at δ 2.17 corresponded to the acetoxyl group, the remaining multiplet at δ 3.17-3.21 ascribe to CHOAc proton.

On the basis of above result **At-1** was characterized as a new triterpene named as **Lupan-3-ol,12,20 diene (I)**



(I)

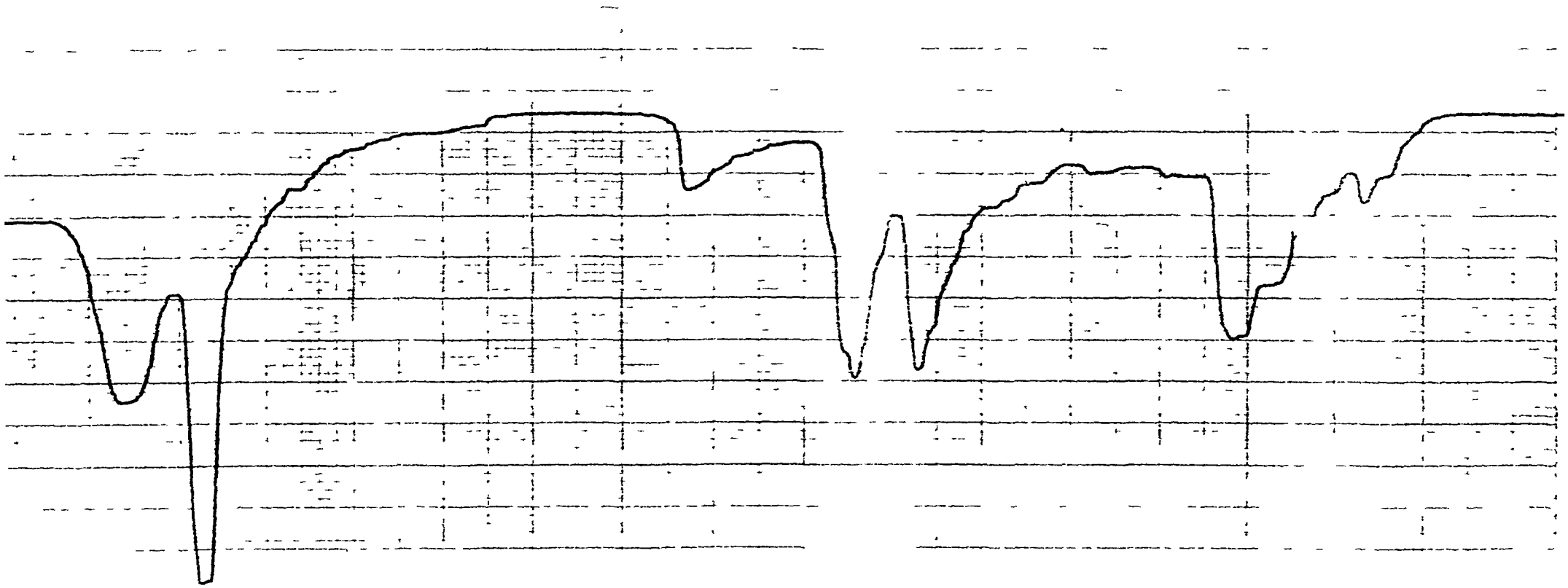
(Table-1)

^{13}C-NMR spectral data of At-1

Assignment	Signals	Assignment	Signals
C-1	35.52	C-16	33.27
C-2	25.09	C-17	48.25
C-3	78.88	C-18	51.15
C-4	37.31	C-19	55.44
C-5	55.25	C-20	150.90
C-6	18.26	C-21	27.37
C-7	34.23	C-22	38.35
C-8	39.94	C-23	31.27
C-9	50.38	C-24	19.25
C-10	37.64	C-25	16.63
C-11	20.87	C-26	16.05
C-12	129.63	C-27	15.34
C-13	142.68	C-28	17.94
C-14	47.92	C-29	109.25
C-15	29.76	C-30	21.04

Spectrum run at 300MHz in $CDCl_3$

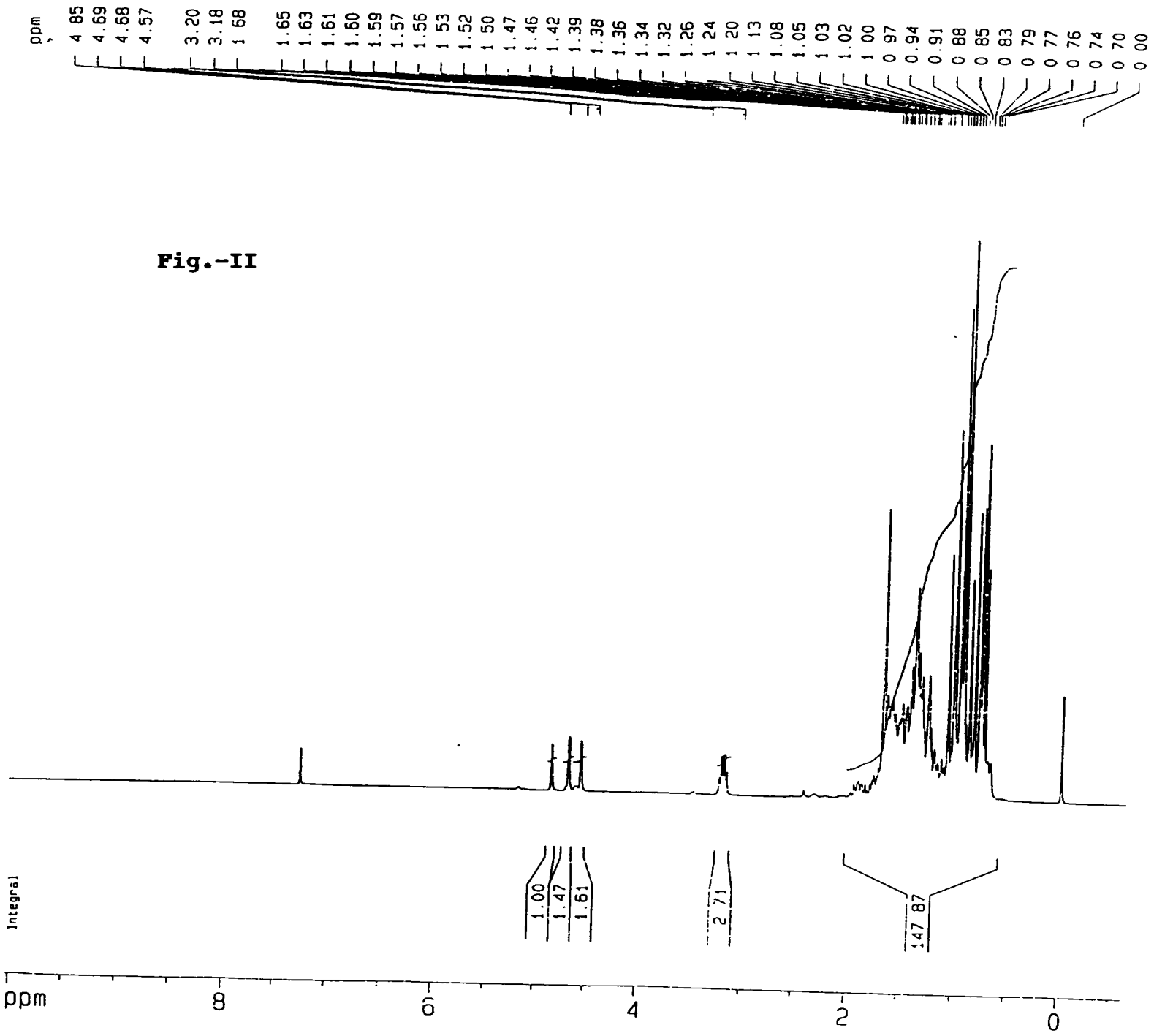
Fig.-I



AT-1-Pet KBr →

I-S6 8 mts Prof. M. Iqbal Lah 6/4/2000

Fig.-II



Current Data Parameters

NAME	
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20000629
Time	15 54
INSTRUM	GMX300
PROBHD	5 mm Multinu
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	0
SWH	5995 204 Hz
FIDRES	0 182959 Hz
AQ	2 7323011 sec
RG	128
DW	83 400 usec
DE	6 00 usec
TE	298 0 K
D1	1 00000000 sec

---------- CHANNEL f1 ----------

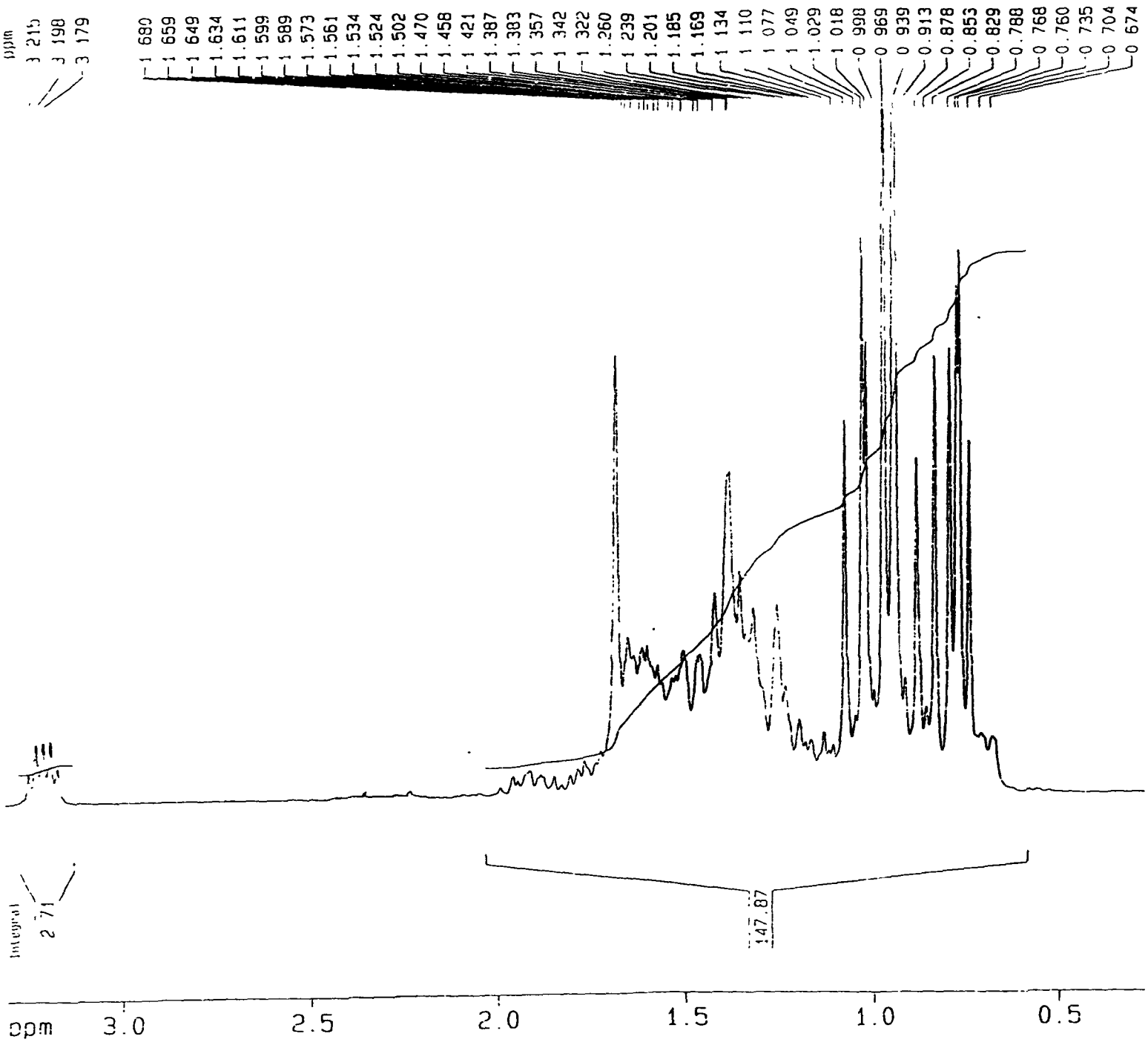
NUC1	1H
P1	6 88 usec
PL1	-3 00 dB
SFO1	300 1314084 MHz

F2 - Processing parameters

SI	16384
SF	300 1300054 MHz
WDW	EM
SSB	0
LB	0 30 Hz
GB	0
PC	1 00

1D NMR plot parameters

CX	20 00 cm
F1P	10 023 ppm
F1	3008 05 Hz
F2P	-0 628 ppm
F2	-188 47 Hz
PPMCM	0.53252 ppm/cm
HZCM	159 82637 Hz/cm



Current Data Parameters

NAME	
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20000629
Time	15 54
INSTRU	DRX300
PROBHD	5 mm Multinu
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	0
SWH	5995 204 Hz
FIDRES	0 182959 Hz
AQ	2 7329011 sec
RG	128
DW	83 400 usec
DE	6 00 usec
TE	298 0 K
D1	1 00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	6 88 usec
PL1	-3 00 dB
SFO1	300.1314084 MHz

F2 - Processing parameters

SI	16384
SF	300.1300054 MHz
WDW	EM
SSB	0
LB	0 30 Hz
GB	0
PC	1 00

1G NMR plot parameters

CX	20.00 cm
F1P	3.296 ppm
F1	989 20 Hz
F2P	0 274 ppm
F2	82 17 Hz
PPMCM	0.15111 ppm/cm
HZCM	45.35119 Hz/cm

केo औo अनुo संo
C. D. R. I.

CENTRAL DRUG RESEARCH INSTITUTE
06-27-2000

JN2703X.LRP AT-1-PET/MR H MUHAISEN/AMU #2284
Date run : 06-27-2000 Operator : PRAKASH/A.SONI/SUNIL

Scan : 27 RT= 3: 1 No.ions= 152 Base= 5.6%F TIC= 49633
NO Peaks After 500

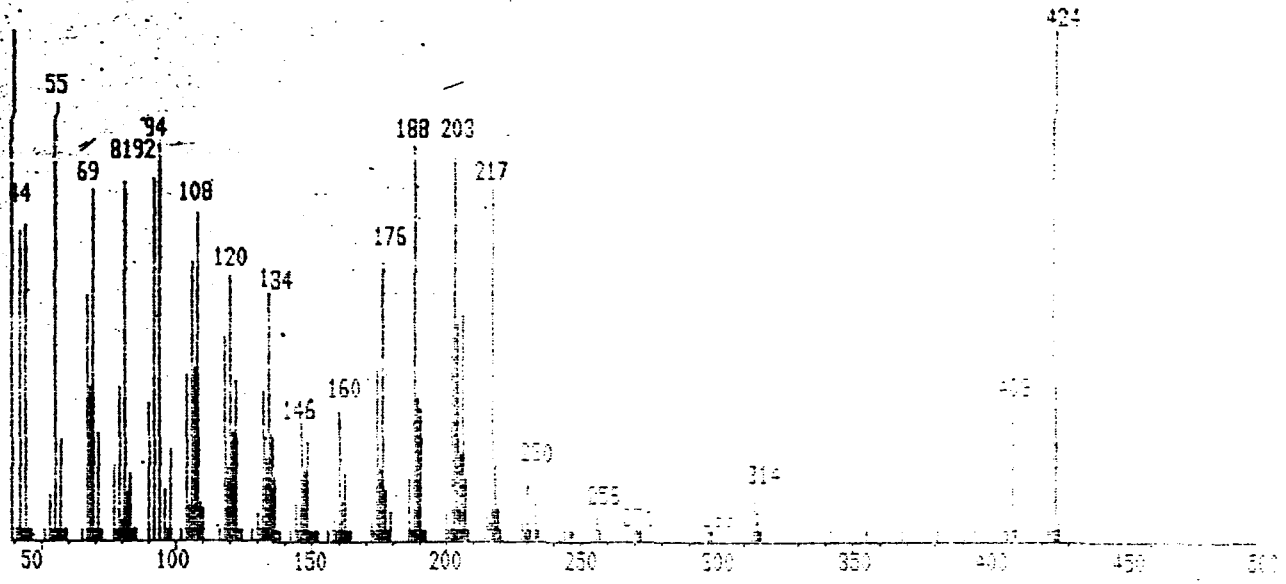
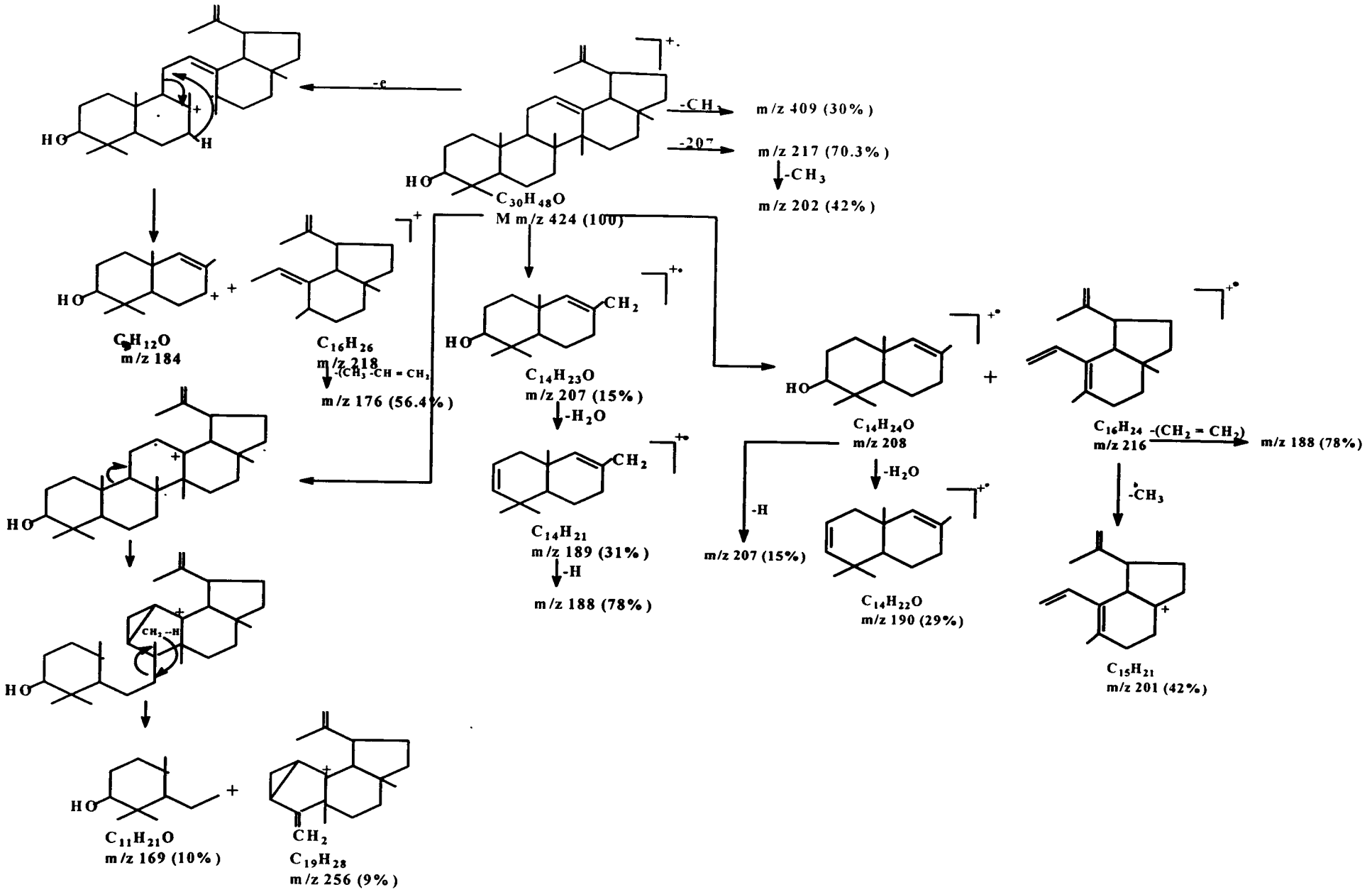


Fig.-III


HO
-e
-CH3
m/z 409 (30%)
-207
m/z 217 (70.3%)
-CH3
m/z 202 (42%)
C30H48O
M m/z 424 (100)
C12H20O
m/z 184
C16H26
m/z 218
-(CH3-CH=CH2)
m/z 176 (56.4%)
CH2
C14H23O
m/z 207 (15%)
-H2O
C14H21
m/z 189 (31%)
-H
m/z 188 (78%)
C14H24O
m/z 208
-H2O
C14H22O
m/z 190 (29%)
-H
m/z 207 (15%)
C16H24
m/z 216
-(CH2=CH2)
m/z 188 (78%)
-CH3
C15H21
m/z 201 (42%)
CH3-H
C11H21O
m/z 169 (10%)
CH2
C19H28
m/z 256 (9%)

(Scheme-I)

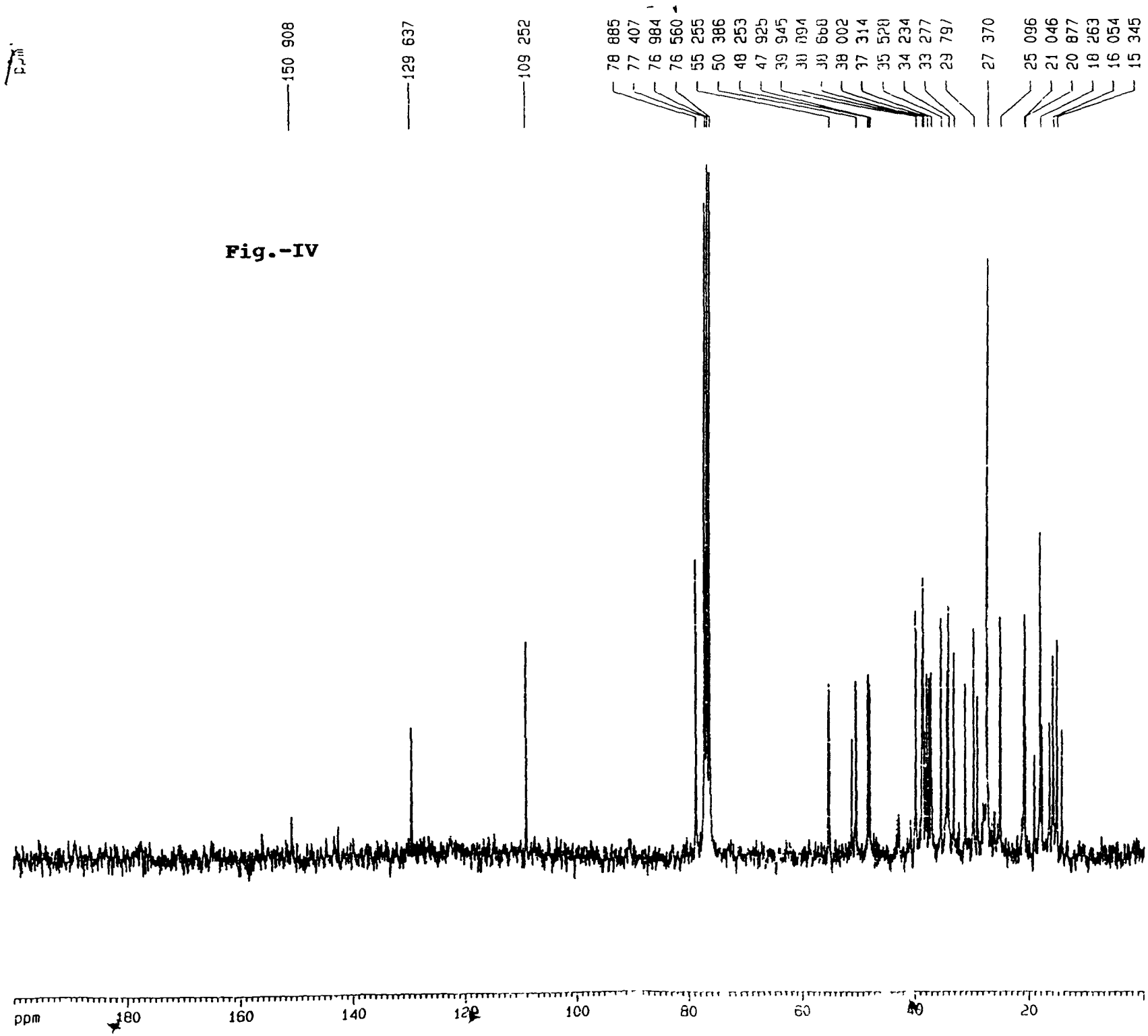


Fig.-IV

Current Data Parameters

NAME	c13
EXPNO	155
PROCNO	1

F2 - Acquisition Parameters

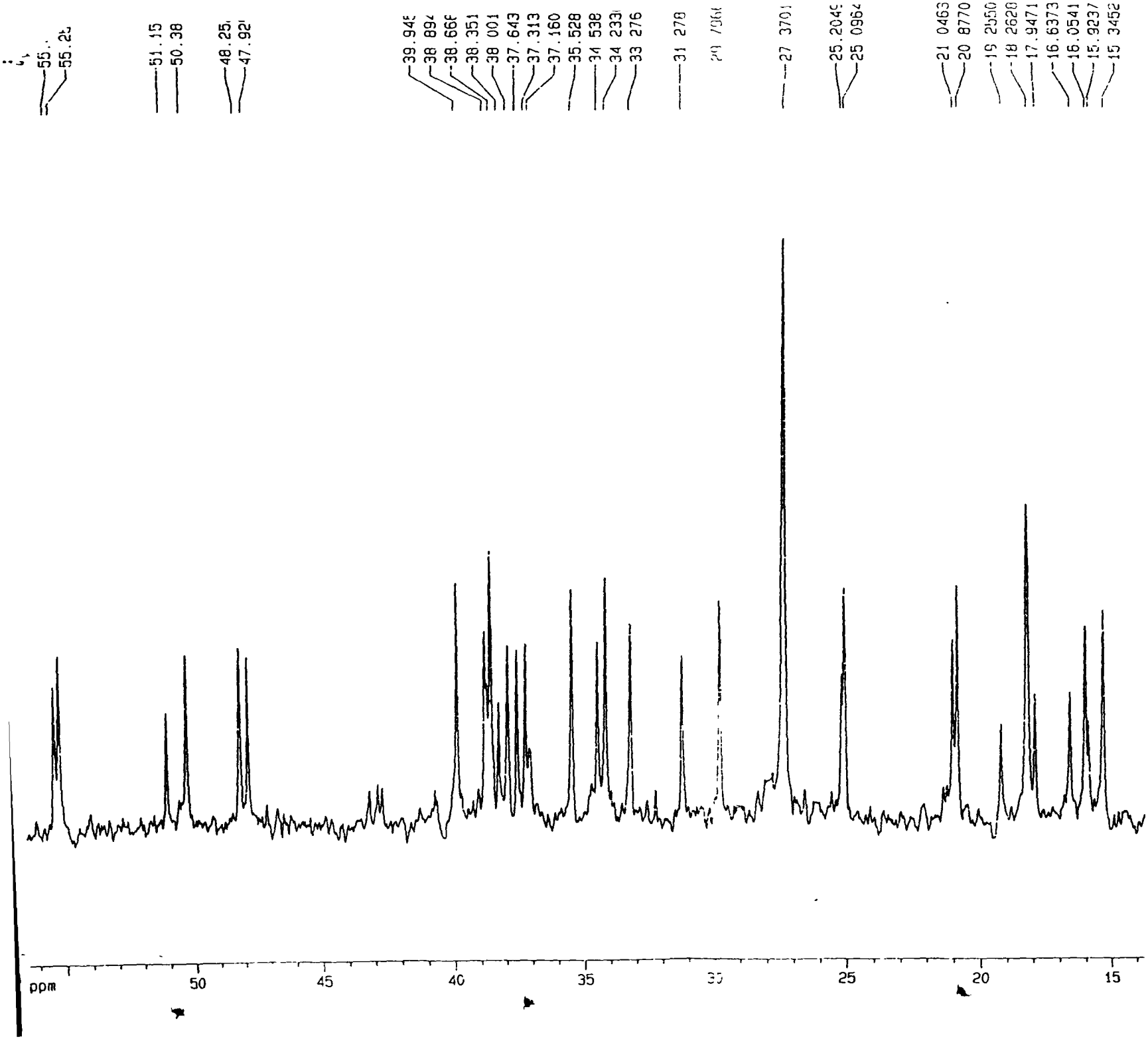
Date_	500000
Time	14 59
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zgdc
TD	16384
SOLVENT	CDCl3
NS	11596
DS	0
SWH	16832 393 Hz
FIDRES	1 149438 Hz
AQ	0 4350452 sec
RG	4597 6
DW	26 550 usec
DE	4 50 usec
TE	300 0 K
d11	0 0300000 sec
PL12	18 00 dB
CPDPRG2	waltz16
PCPD2	100 00 usec
SFO2	300 1330013 MHz
NUC2	1H
PL2	-6 00 dB
D1	1 00000000 sec
P1	5 50 usec
DE	4 50 usec
SFO1	75 4767751 MHz
NUC1	13C
PL1	-6 00 dB

F2 - Processing parameters

SI	16384
SF	75 4677526 MHz
WDW	EM
SSB	0
LB	5 00 Hz
GB	0
PC	0 60

1D NMR plot parameters

CX	22 00 cm
F1P	200 000 ppm
F1	15093 55 Hz
F2P	0 000 ppm
F2	0 00 Hz
PPMCM	9 09091 ppm/cm
HZCM	686 07043 Hz/cm



Current Data Parameters

NAME	c13
EXPNO	155
PROCNO	1

F2 - Acquisition Parameters

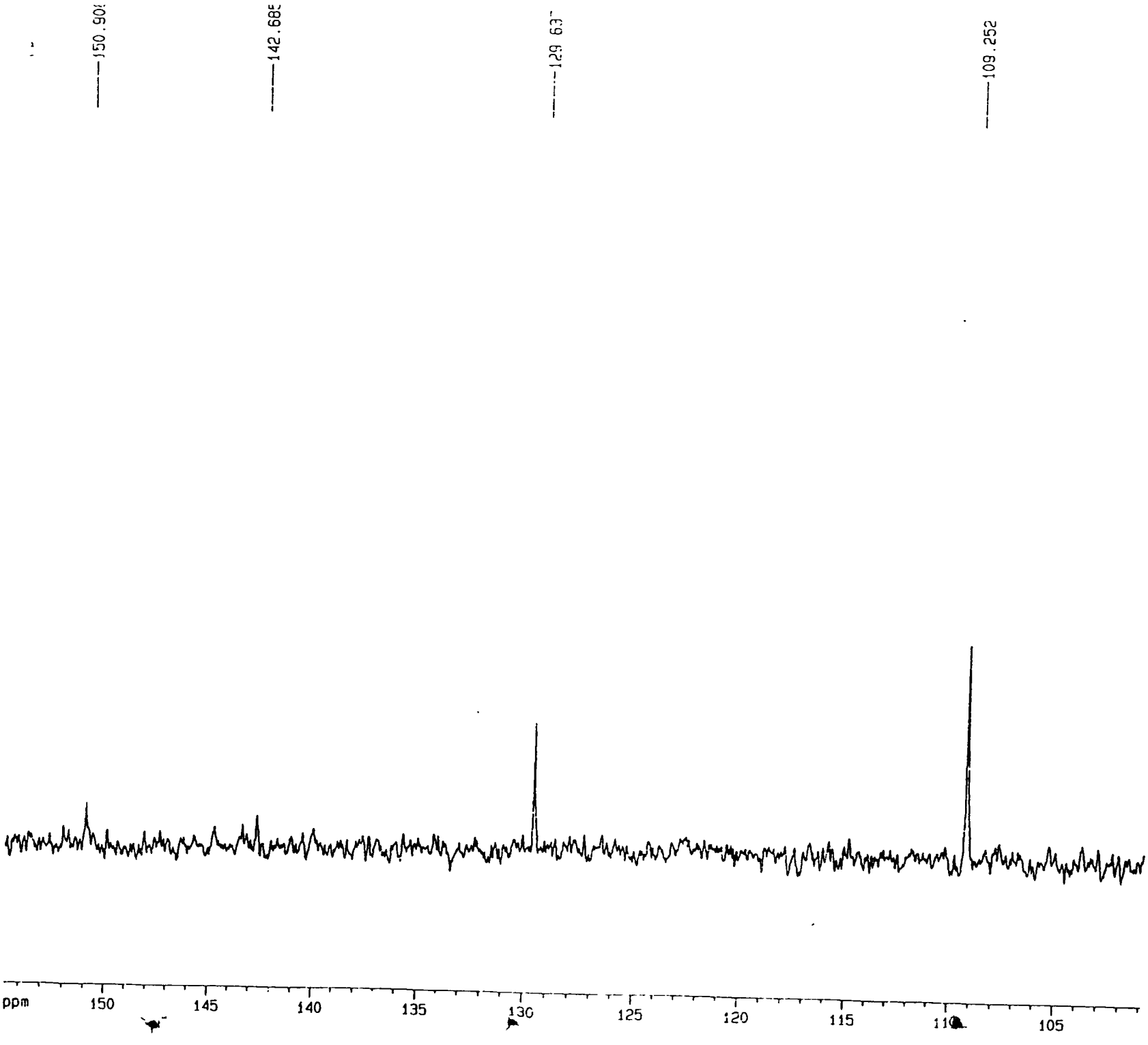
Date_	500000
Time	14.59
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zgdc
TD	16384
SOLVENT	CDCl3
NS	11596
DS	0
SWH	18832.393 Hz
FIDRES	1.149438 Hz
AQ	0.4350452 sec
RG	4597.6
DW	26.560 usec
DE	4.50 usec
TE	300.0 K
d11	0.0300000 sec
PL12	18.00 dB
CPDPRG2	waltz16
PCPD2	100.00 usec
SFO2	300.1330013 MHz
NUC2	1H
PL2	-6 00 dB
D1	1.00000000 sec
P1	5.50 usec
DE	4.50 usec
SFO1	75.4767751 MHz
NUC1	13C
PL1	-6.00 dB

F2 - Processing parameters

SI	16384
SF	75 4677526 MHz
WDW	EM
SSB	0
LB	5 00 Hz
GB	0
PC	0.60

1D NMR plot parameters

CX	22 00 cm
F1P	56 472 ppm
F1	4261 78 Hz
F2P	13 721 ppm
F2	1035 47 Hz
PPMCM	1 94322 ppm/cm
HZCM	146 65074 Hz/cm



Current Data Parameters

NAME	c13
EXPNO	155
PROCNO	1

F2 - Acquisition Parameters

Date_	500000
Time	14.59
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zgdc
TD	16384
SOLVENT	CDCl3
NS	11596
DS	0
SWH	18832.393 Hz
FIDRES	1.149438 Hz
AQ	0.4350452 sec
RG	4597.6
DW	26.550 usec
DE	4.50 usec
TE	300.0 K
d11	0.0300000 sec
PL12	18.00 dB
CPDPRG2	waltz16
PCPD2	100.00 usec
SFO2	300.1330013 MHz
NUC2	1H
PL2	-6.00 dB
D1	1.00000000 sec
P1	5.50 usec
DE	4.50 usec
SFO1	75.4767751 MHz
NUC1	13C
PL1	-6.00 dB

F2 - Processing parameters

SI	16384
SF	75.4677526 MHz
WDW	EM
SSB	0
LB	5.00 Hz
GB	0
PC	0.50

1D NMR plot parameters

CX	22.00 cm
F1P	154.661 ppm
F1	11671.93 Hz
F2P	100.862 ppm
F2	7611.84 Hz
PPMCM	2.44541 ppm/cm
HZCM	184.[illegible]951 Hz/cm

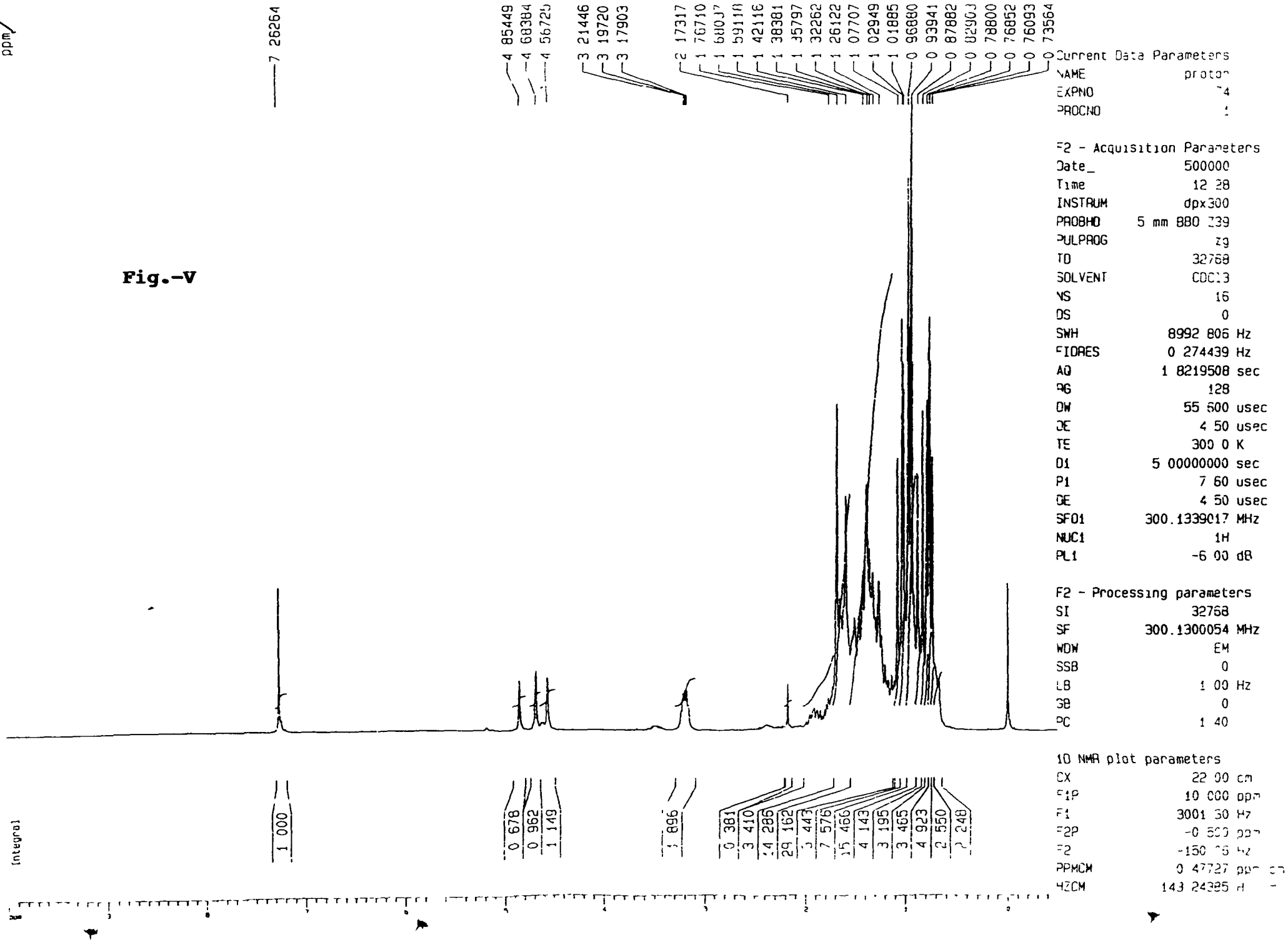
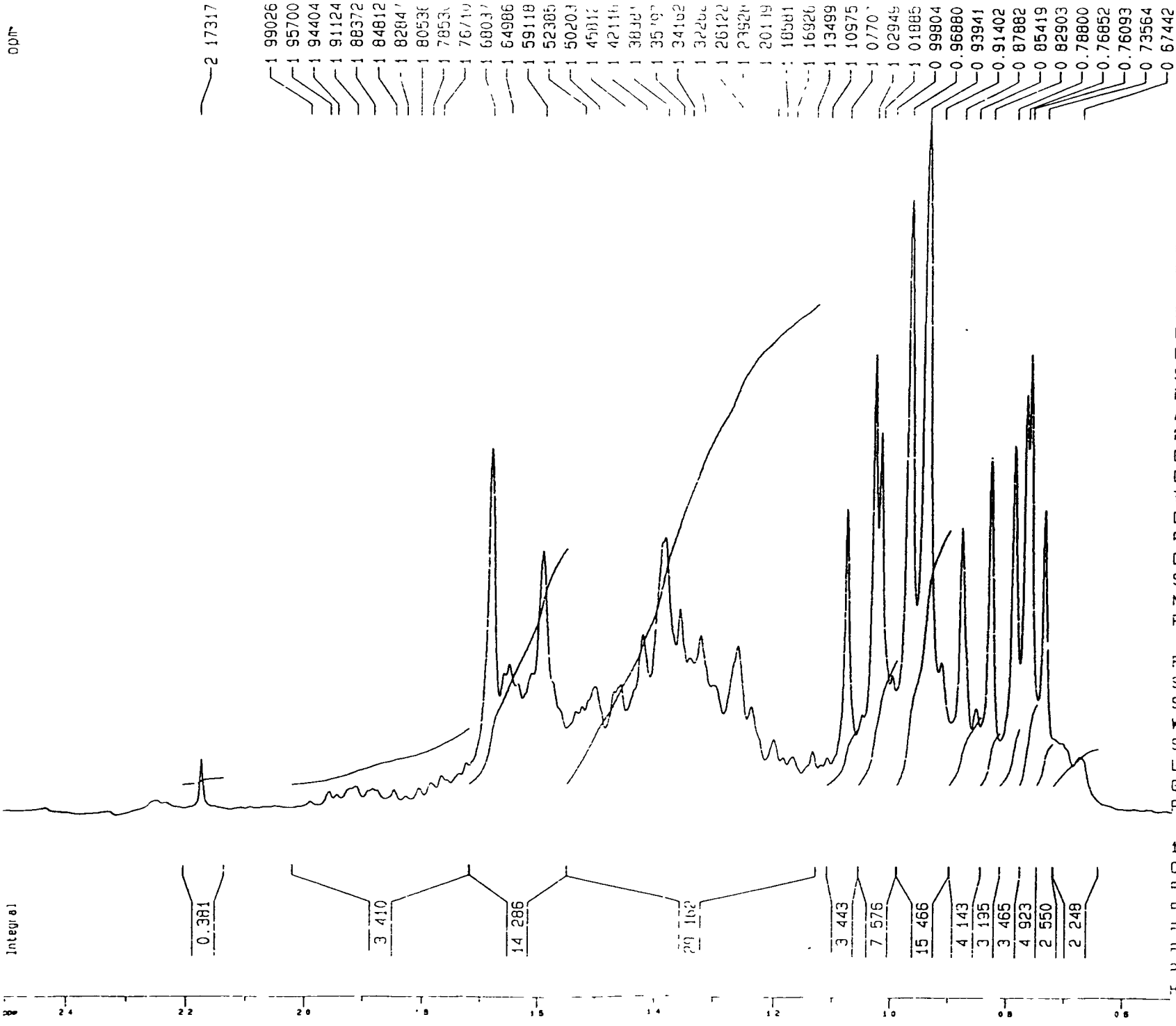

ppm
7.26264
4.85449
4.68384
4.56725
3.21446
3.19720
3.17903
2.17317
1.76710
1.59118
1.42116
1.38381
1.35797
1.32262
1.26122
1.07707
1.02949
1.01885
0.96880
0.93941
0.87882
0.78800
0.76852
0.76093
0.73564
Current Data Parameters
NAME proton
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 500000
Time 12 28
INSTRUM dpx300
PROBHD 5 mm BBO 239
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 8992.806 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 128
DW 55.600 usec
DE 4.50 usec
TE 300.0 K
D1 5.00000000 sec
P1 7.60 usec
GE 4.50 usec
SFO1 300.1339017 MHz
NUC1 1H
PL1 -6.00 dB
F2 - Processing parameters
SI 32768
SF 300.1300054 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 22.00 cm
F1P 10.000 ppm
F1 3001.30 Hz
F2P -0.800 ppm
F2 -150.76 Hz
PPMCM 0.47727 ppm/cm
HZCM 143.24385 Hz/cm
Integral
1.000
0.678
0.962
1.149
3.896
0.381
3.410
14.286
29.162
5.443
7.576
15.466
4.143
3.195
3.465
4.923
2.550
2.248

Fig.-V



Current Data Parameters

NAME	proton
EXPNO	74
PROCNO	1

F2 - Acquisition Parameters

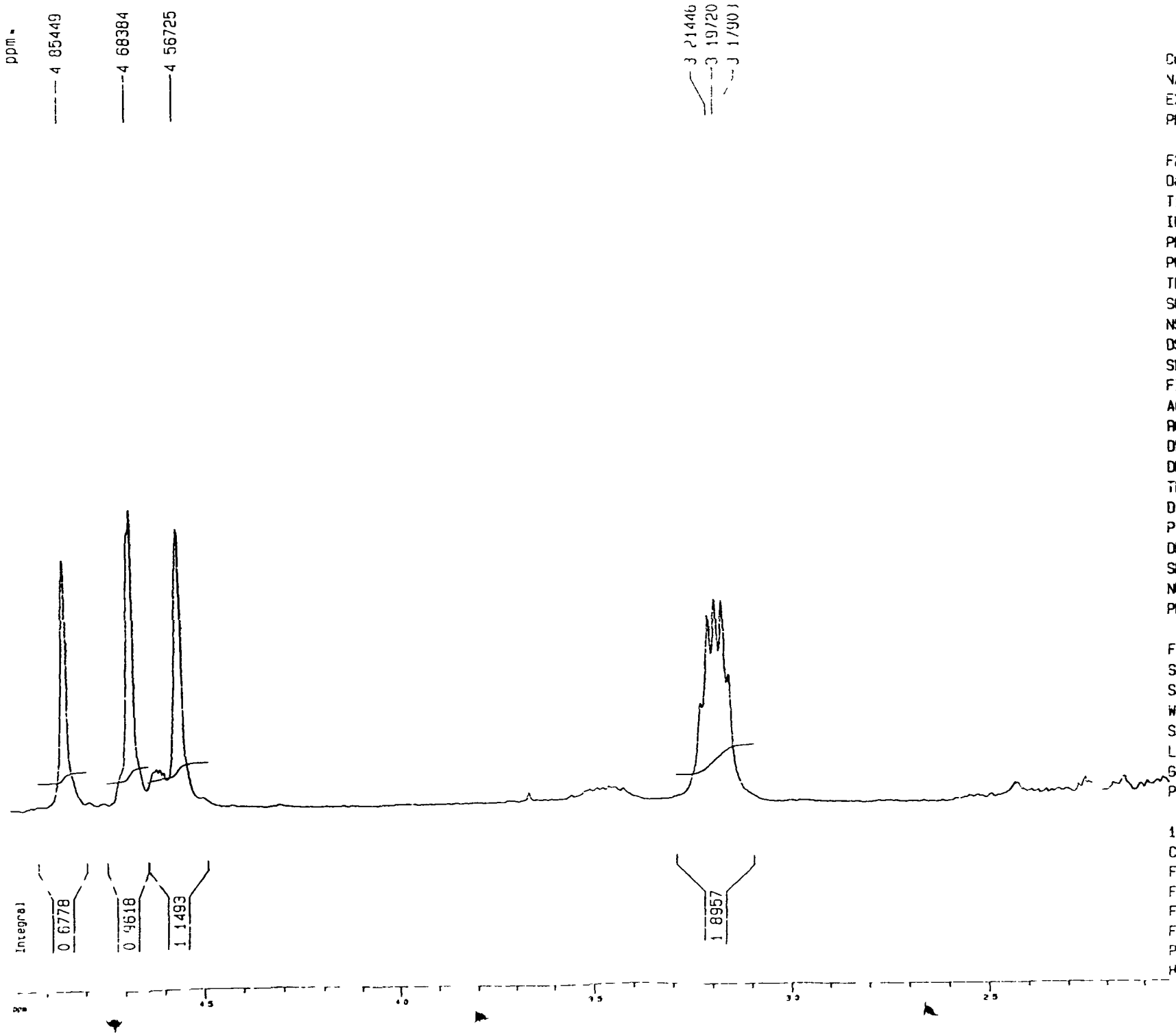
Date_	500000
Time	12 28
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	0
SWH	8992.806 Hz
FIDRES	0.274439 Hz
AQ	1 8219508 sec
RG	128
DW	55.600 usec
DE	4.50 usec
TE	300.0 K
D1	5 00000000 sec
P1	7.60 usec
DE	4.50 usec
SFO1	300.1339017 MHz
NUC1	1H
PL1	-6.00 dB

F2 - Processing parameters

SI	32768
SF	300 1300054 MHz
WDW	EM
SSB	0
LB	1 00 Hz
GB	0
PC	1 40

1D NMR plot parameters

CX	22 00 cm
F1P	2 503 ppm
F1	751 27 Hz
F2P	0 517 ppm
F2	155 22 Hz
PPMCM	0 09027 ppm/cm
HZCM	27 09322 Hz/cm



Current Data Parameters

NAME	proton
EXPNO	74
PROCNO	1

F2 - Acquisition Parameters

Date_	500000
Time	12 28
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	0
SWH	8992 806 Hz
FIDRES	0 274439 Hz
AQ	1 8219508 sec
RG	128
DW	55 600 usec
DE	4 50 usec
TE	300 0 K
D1	5 00000000 sec
P1	7 60 usec
DE	4 50 usec
SFO1	300 1339017 MHz
NUC1	1H
PL1	-6 00 dB

F2 - Processing parameters

SI	32768
SF	300 1300054 MHz
WDW	EM
SSB	0
LB	1 00 Hz
GB	0
PC	1 40

1D NMR plot parameters

CX	22 00 cm
F1P	4 991 ppm
F1	1498 06 Hz
F2P	2 035 ppm
F2	610 78 Hz
PPMCM	0 13438 ppm/cm
HZCM	40 33054 Hz/cm

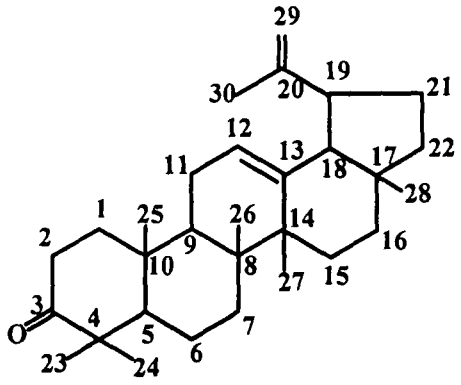
At-2:

The compound **(At-2)** obtained from the column with petrol-benzene (7:3), was crystallized with chloroform-methanol and afforded white shining crystals (50 mg) m.p.195^{0}C, analyzed for $C_{30}H_{46}O$. It gave positive Leiberman-Buchard[14] and Nollers test[8] indicating the presence of a triterpene. A yellow colour with tetranitromethane showed the presence of double bond. It gave a positive test to Zimmerman reaction[10] (a violet colour with m-dinitrobenzene in caustic potash) indicating the presence of keto group at 3-position which was further confirmed by its **ir** spectrum (**Fig.-VI**), which showed the characteristic bands at 1700 cm^{-1} (C=O), 1450 cm^{-1} (C=C), 1375 cm^{-1} (geminal dimethyl) and 895 cm^{-1} (terminal methylene).

The ^{1}**H-nmr** spectrum **(Fig.-VII)** of the compound **(At-2)** showed 7-methyl groups at δ 0.79 (3H), 0.94 (6H), 1.02 (3H), 1.03 (3H), 1.05 (3H) and 1.68 (3H). The CH_2 protons appeared in the range of δ 1.3-1.57. The double bond at Δ^{12} position was exhibited by the singlet at δ 4.86 and terminal methylene group was centered at δ 4.52 and 4.57. The ^{13}**C-nmr** spectrum (**Fig.-VIII**) exhibited the presence of thirty carbons, the carbonyl group appeared at 206.1, assignment of other carbons is given in **(Table-2)**.

The **mass** spectrum **(Fig.-IX)** of **At-2** showed the molecular ion peak at m/z 422, other fragments are rationalized by the **(Scheme-II)**.

On the basis of the above discussion the compound **(At-2)** was characterized as a novel triterpene **Lup-12,20-dien 3-one (II).**



(II)

The above assigned structure (II) was further confirmed by the bromination of Lupan-3-ol, 12,20 diene (I) followed by Jones oxidation and debromination. The final product so obtained was found to be completely similar with that of **Lup-12,20-dien 3-one** (m.p., m.m.p, R_f value and co-TLC)

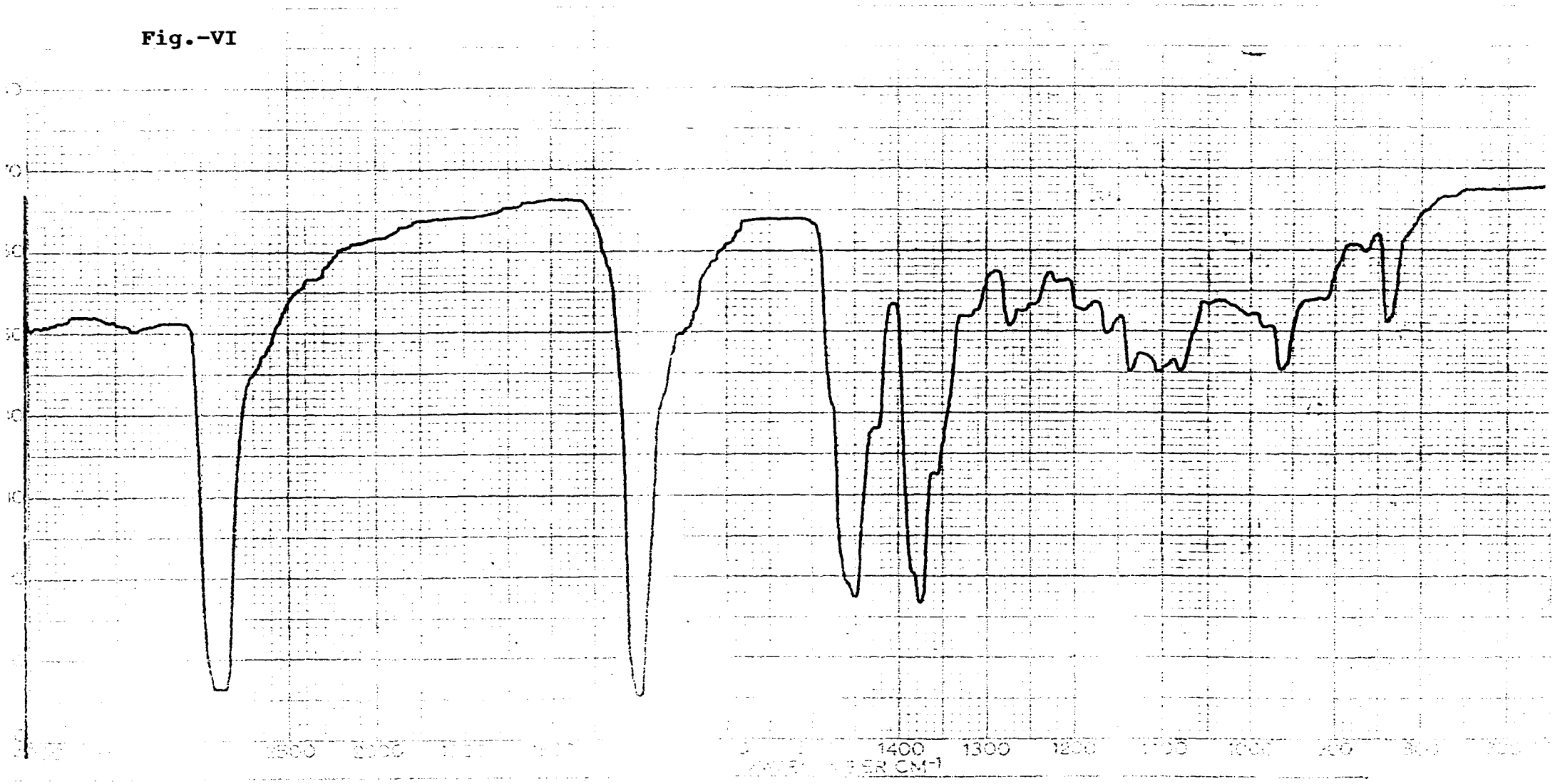
(Table-2)

^{13}C-NMR spectral data of At-2

Assignment	Signals	Assignment	Signals
C-1	38.52	C-16	33.32
C-2	25.27	C-17	43.35
C-3	206.1	C-18	46.80
C-4	37.62	C-19	40.64
C-5	54.88	C-20	123.45
C-6	19.68	C-21	27.48
C-7	33.81	C-22	39.30
C-8	39.85	C-23	31.30
C-9	50.50	C-24	21.68
C-10	37.36	C-25	15.93
C-11	20.92	C-26	16.54
C-12	129.86	C-27	14.46
C-13	142.55	C-28	23.66
C-14	47.25	C-29	121.51
C-15	29.18	C-30	26.21

Spectrum run at 300MHz in $CDCl_3$

Fig.-VI



AT-5-Pct KBr

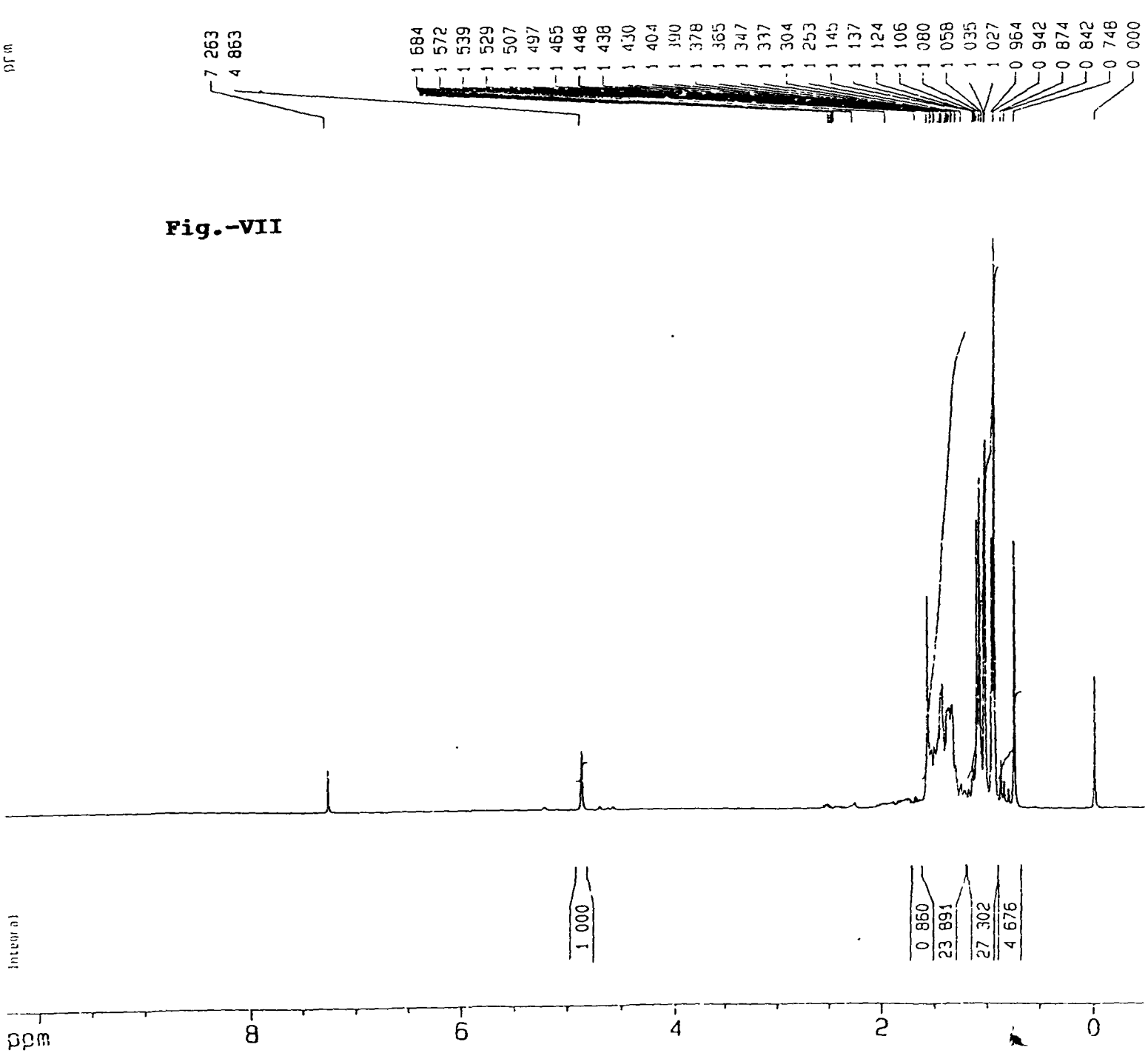
AT-11

EMARKS

Prof. M. Ilyas Lah

16-3-2000

Fig.-VII



Current Data Parameters

NAME	
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20000629
Time	15 59
INSTRUM	DRX300
PROBHD	5 mm Multinu
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	0
SWH	5995 204 Hz
FIDRES	0 182959 Hz
AQ	2 7329011 sec
RG	181
DW	83 400 usec
DE	6 00 usec
TE	298 0 K
D1	1 00000000 sec

========== CHANNEL f1 ==========

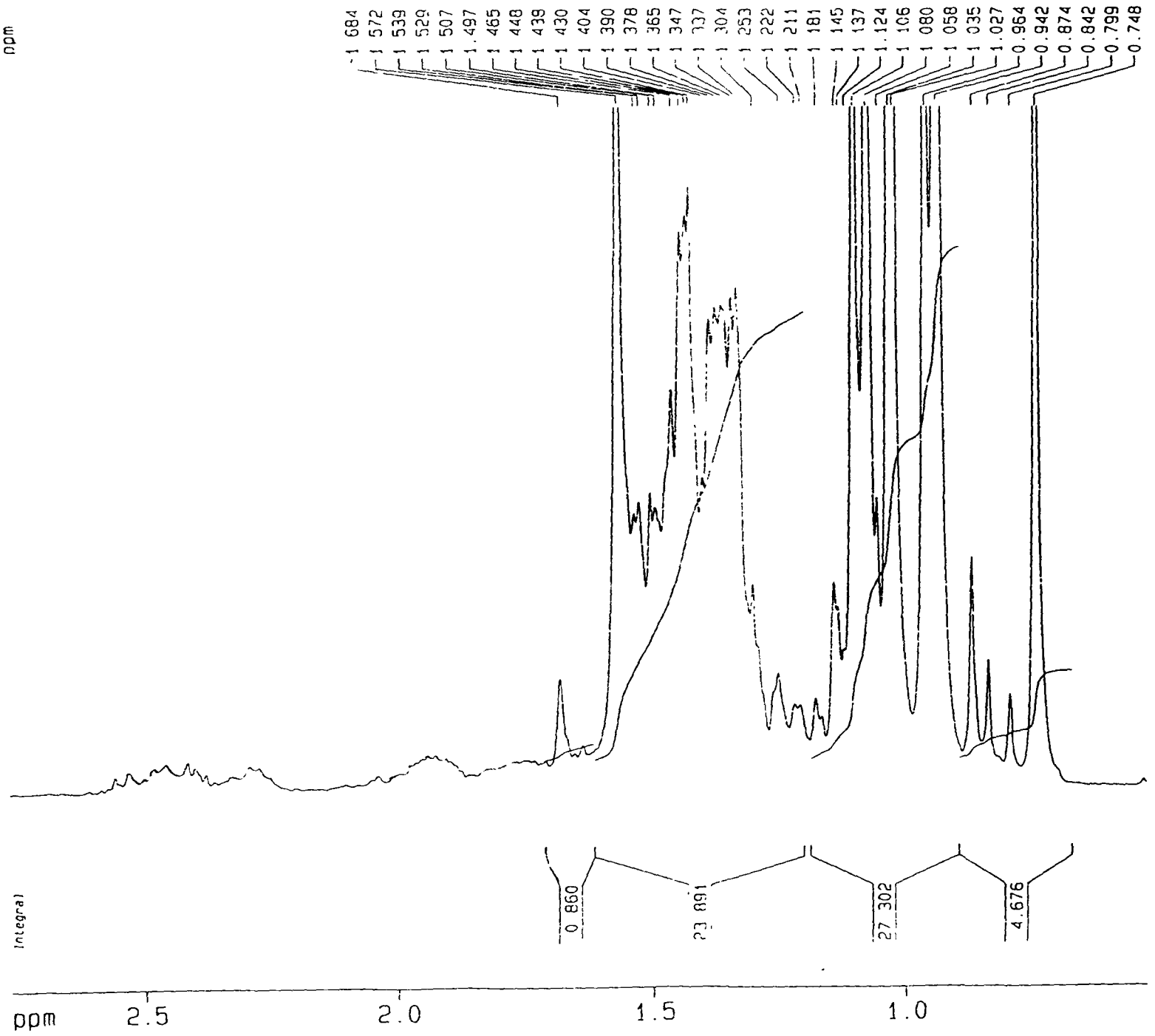
NUC1	1H
P1	6 88 usec
PL1	-3 00 dB
SFO1	300 1314084 MHz

F2 - Processing parameters

SI	16384
SF	300 1300054 MHz
WDW	EM
SSB	0
LB	0 30 Hz
GB	0
PC	1 00

1D NMR plot parameters

CX	20 00 cm
F1P	10 320 ppm
F1	3097 23 Hz
F2P	-0 468 ppm
F2	-140 46 Hz
PPMCM	0 53938 ppm/cm
HZCM	161 88422 hz/cm



Current Data Parameters

NAME	
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20000629
Time	15.59
INSTRUM	DRX300
PROBHD	5 mm Multinu
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	0
SWH	5995.204 Hz
FIDRES	0.182959 Hz
AQ	2.7329011 sec
RG	181
DW	83.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	6.88 usec
PL1	-3.00 dB
SFO1	300.1314084 MHz

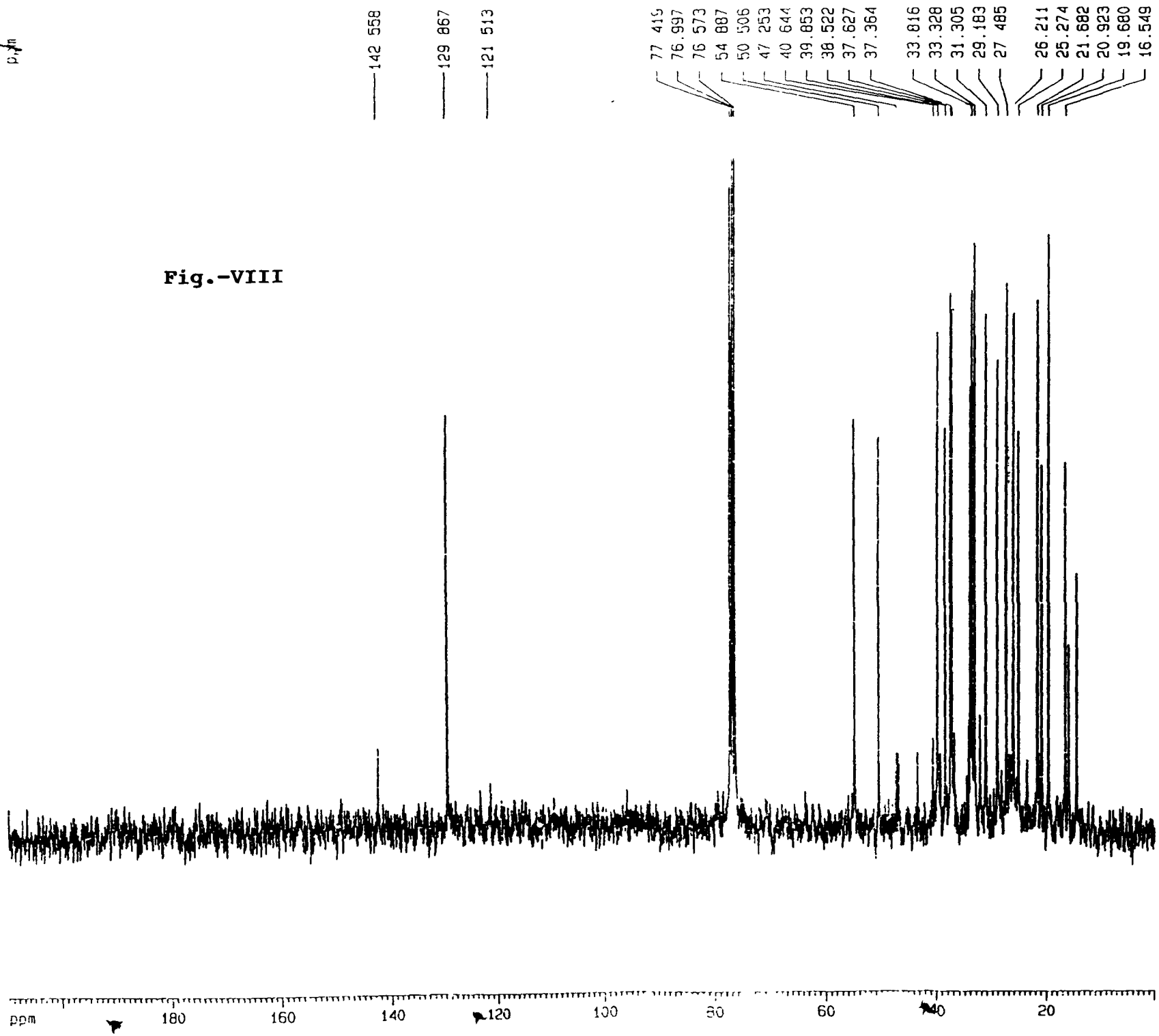
F2 - Processing parameters

SI	16384
SF	300.1300054 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	2.766 ppm
F1	830.11 Hz
F2P	0.532 ppm
F2	159.60 Hz
PPMCM	0.11170 ppm/cm
HZCM	33.52522 Hz/cm

Fig.-VIII



Current Data Parameters

NAME	c13
EXPNO	156
PROCNO	1

F2 - Acquisition Parameters

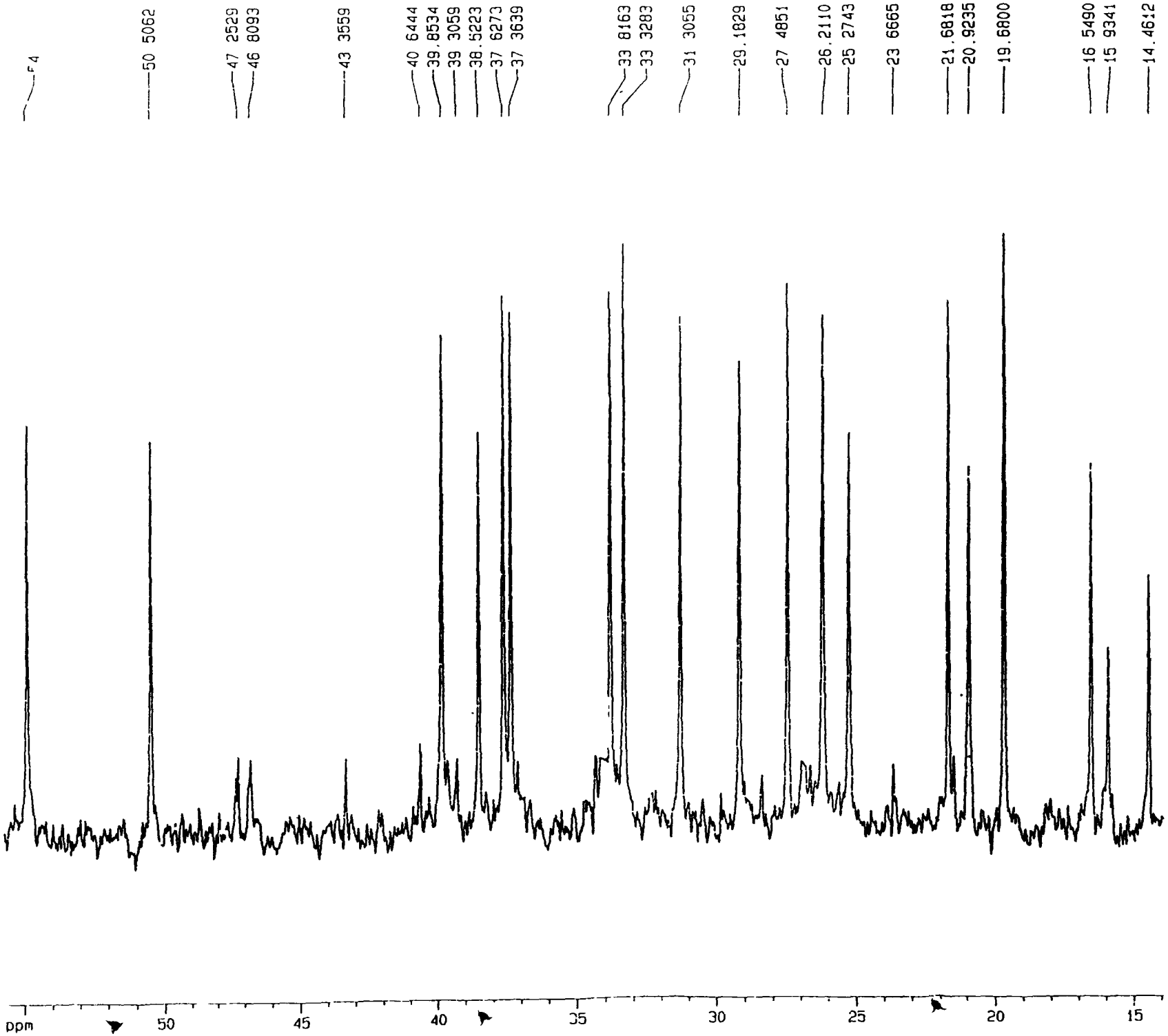
Date_	500000
Time	16.12
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zgdc
TD	16384
SOLVENT	CDCl3
NS	8917
DS	0
SWH	18832 393 Hz
FIDRES	1.149438 Hz
AQ	0.4350452 sec
RG	5160.6
DW	26.550 usec
DE	4.50 usec
TE	300.0 K
d11	0.0300000 sec
PL12	18.00 dB
CPDPRG2	waltz16
PCPD2	100.00 usec
SFO2	300.1330013 MHz
NUC2	1H
PL2	-6.00 dB
D1	1.00000000 sec
P1	5.50 usec
DE	4.50 usec
SFO1	75.4767751 MHz
NUC1	13C
PL1	-6.00 dB

F2 - Processing parameters

SI	16384
SF	75.4677491 MHz
WDW	EM
SSB	0
LB	4.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	22.00 cm
F1P	210.000 ppm
F1	15848 23 Hz
F2P	0 000 ppm
F2	0.00 Hz
PPMCM	9 54545 ppm/cm
HZCM	720 37390 Hz/cm



Current Data Parameters

NAME	c13
EXPNO	156
PROCNO	1

F2 - Acquisition Parameters

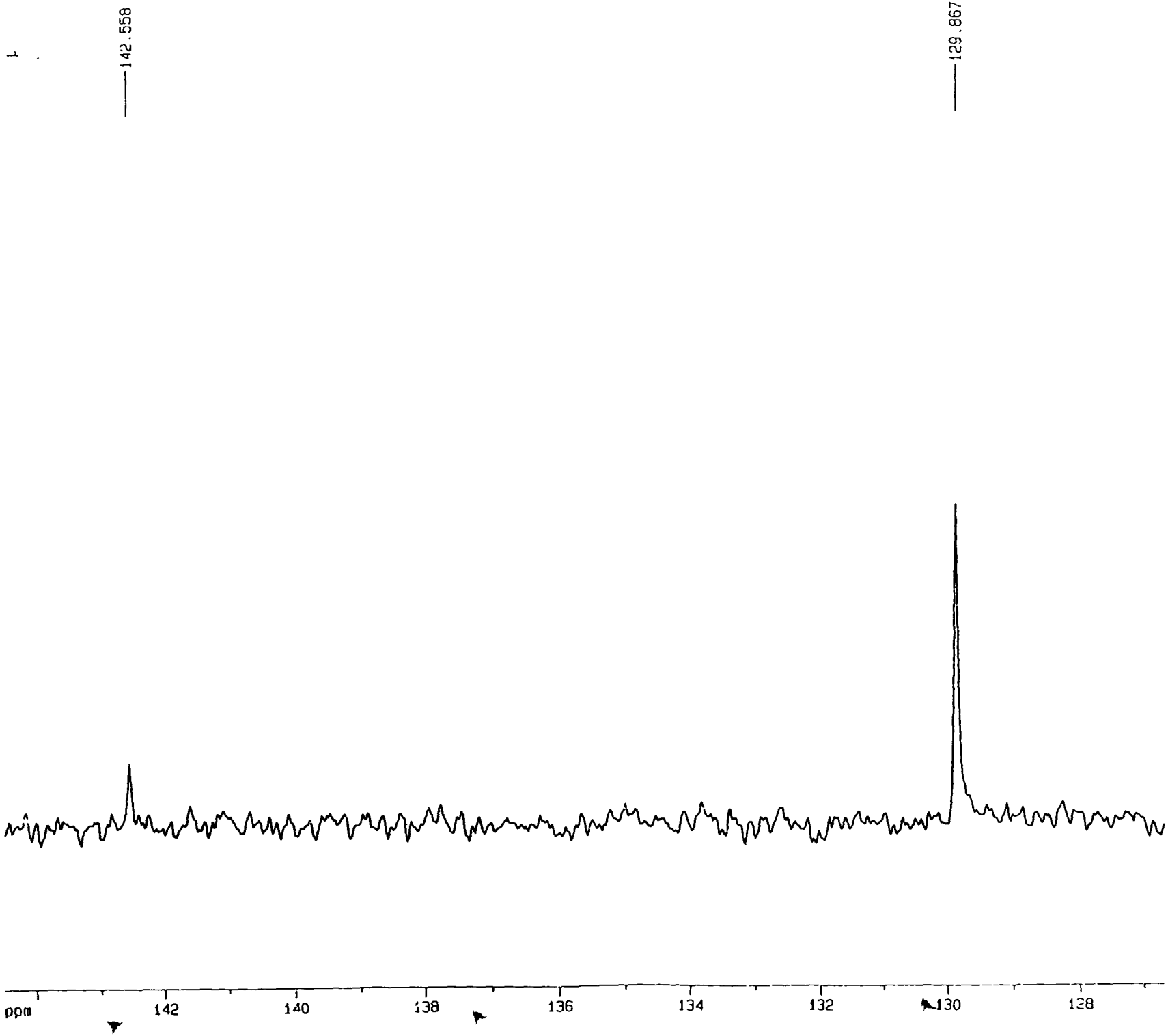
Date_	500000
Time	15 12
INSTRUM	dpx300
PROBHD	5 mm BBO Z39
PULPROG	zgdc
TD	16384
SOLVENT	CDCl3
NS	8917
DS	0
SWH	18832.393 Hz
FIDRES	1.149438 Hz
AQ	0.4350452 sec
RG	5160.6
DW	26.550 usec
DE	4.50 usec
TE	300.0 K
d11	0.0300000 sec
PL12	18.00 dB
CPDPRG2	waltz16
PCPD2	100.00 usec
SFO2	300.1330013 MHz
NUC2	1H
PL2	-6.00 dB
D1	1.00000000 sec
P1	5.50 usec
DE	4.50 usec
SFO1	75.4767751 MHz
NUC1	13C
PL1	-6.00 dB

F2 - Processing parameters

SI	16384
SF	75.4677491 MHz
WDW	EM
SSB	0
LB	4.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	22.00 cm
F1P	55.696 ppm
F1	4203.26 Hz
F2P	13.937 ppm
F2	1051.77 Hz
PPMCM	1.89816 ppm/cm
HZCM	143.24980 Hz/cm



Current Data Parameters

NAME	c13
EXPNO	156
PROCNO	1

F2 - Acquisition Parameters

Date_	500000
Time	16.12
INSTRUM	dpx300
PROBHD	5 mm 88C Z39
PULPROG	zgdc
TD	16384
SOLVENT	CDC13
NS	8917
DS	0
SWH	18832.393 Hz
FIDRES	1.149438 Hz
AQ	0.4350452 sec
RG	5160.6
DW	26.550 usec
DE	4.50 usec
TE	300.0 K
d11	0.0300000 sec
PL12	18.00 dB
CPDPRG2	waltz16
PCPD2	100.00 usec
SFO2	300.1330013 MHz
NUC2	1H
PL2	-6.00 dB
D1	1.00000000 sec
P1	5.50 usec
DE	4.50 usec
SFO1	75.4767751 MHz
NUC1	13C
PL1	-6.00 dB

F2 - Processing parameters

SI	16384
SF	75.4677491 MHz
WDW	EM
SSB	0
LB	4.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

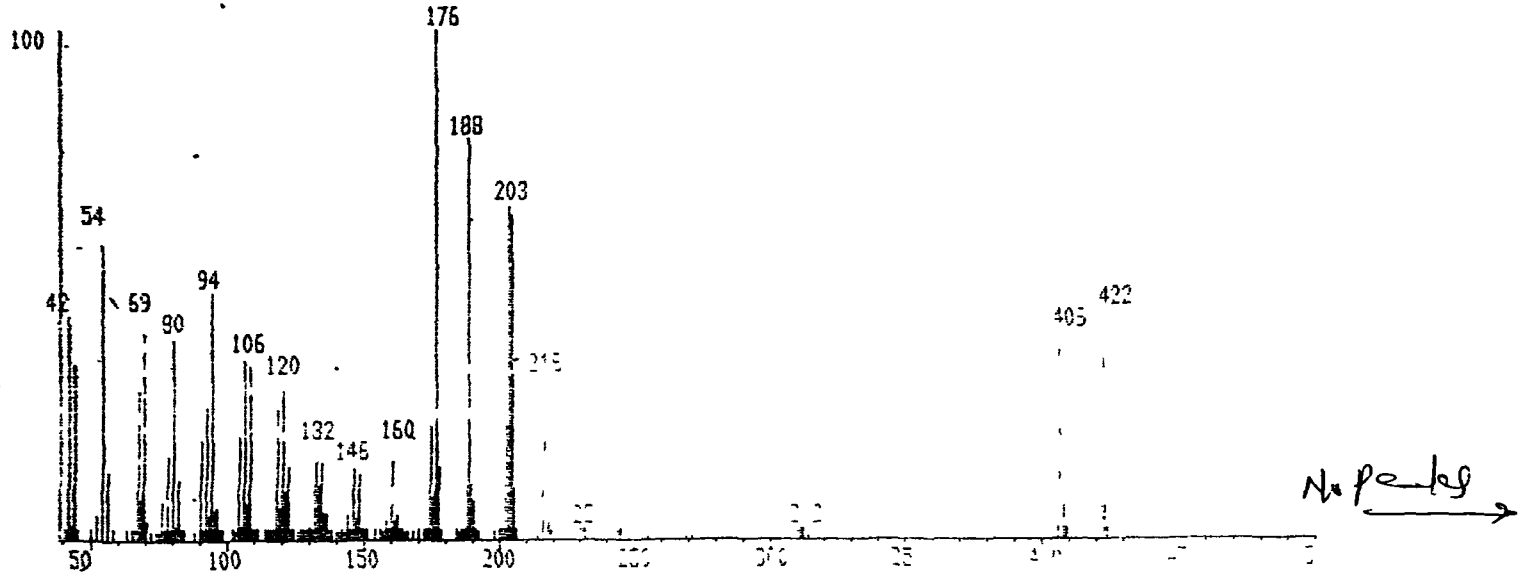
CX	22.00 cm
F1P	144.502 ppm
F1	10905.20 Hz
F2P	126.588 ppm
F2	9560.87 Hz
PPMCM	0.80970 ppm/cm
HZCM	61.10629 Hz/cm

केo ओo अनुo सo
C. D. R. I.

CENTRAL DRUG RESEARCH INSTITUTE
06-27-2000

JN2707X.LRP AT-5-PET/OF H MUHAIEEN/A.U,ALIBAPH/IIIE+
Date run : 06-27-2000 Operator : PPAKASH/A.SONI/SUNIL

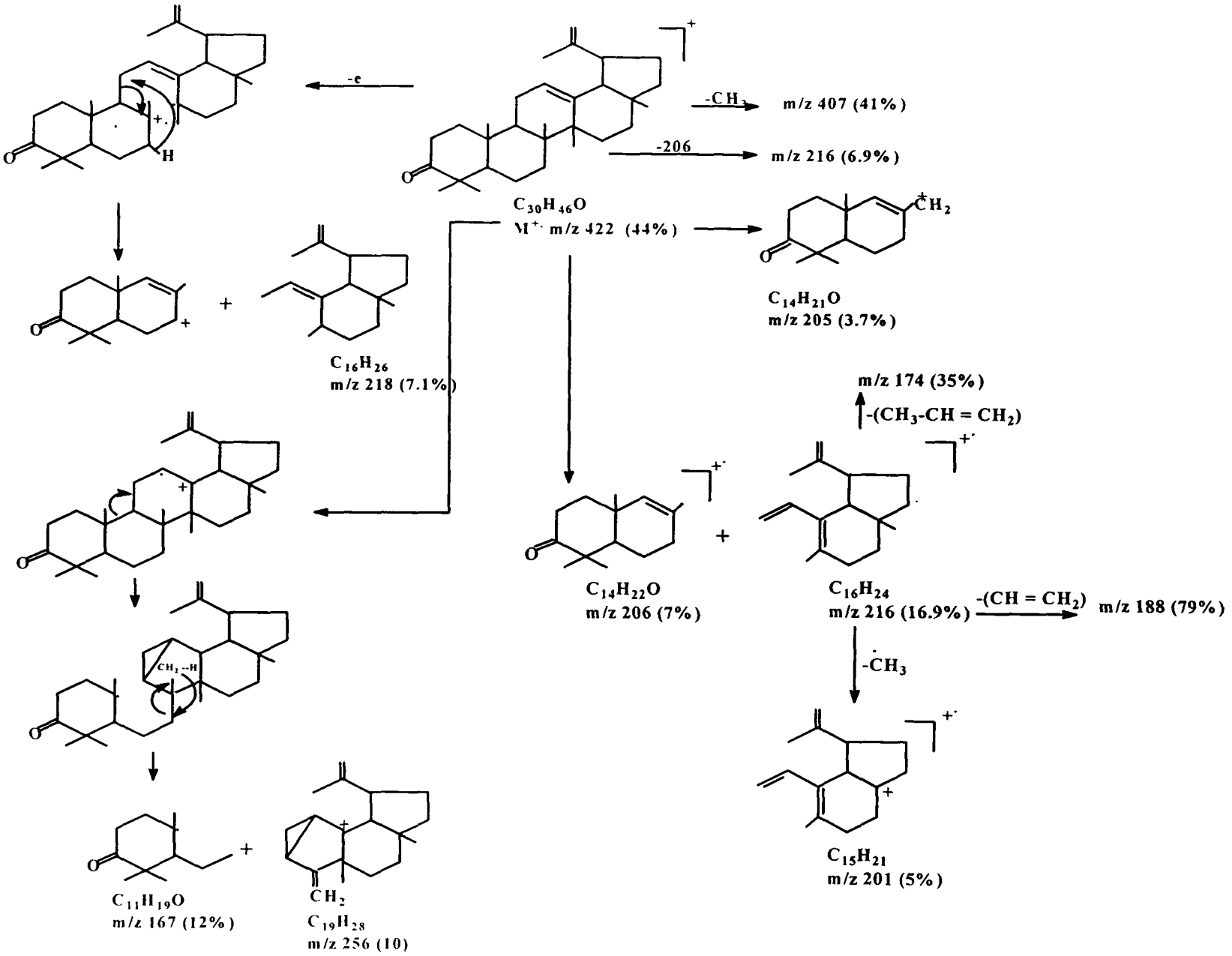
Scan : 7 RT= 0:47 No.ions= 160 Base= 20.7%F TIC=115157



No peaks

.CENTRAL DRUG RESEARCH INSTITUTE
06-27-2000

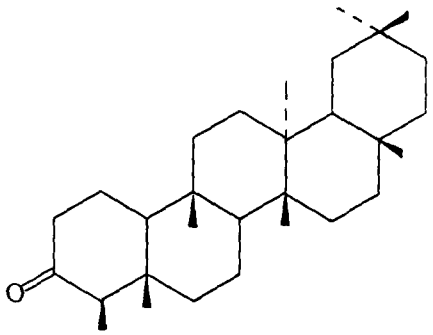
Fig.-IX


-e
-CH3
m/z 407 (41%)
-206
m/z 216 (6.9%)
C30H46O
M+· m/z 422 (44%)
C14H21O
m/z 205 (3.7%)
C16H26
m/z 218 (7.1%)
m/z 174 (35%)
-(CH3-CH = CH2)
C14H22O
m/z 206 (7%)
C16H24
m/z 216 (16.9%)
-(CH = CH2)
m/z 188 (79%)
-CH3
C15H21
m/z 201 (5%)
C11H19O
m/z 167 (12%)
CH2
C19H28
m/z 256 (10)

(Scheme-II)

At-3:

Elution of the column with petrol-benzene (1:1) followed by crystallization from chloroform-methanol gave white needle shaped crystals (150 mg), m.p. 262-64^{0}C. $[\alpha]^{23}_{546}$–29.4^0 ($CDCl_3$). It analysed for $C_{30}H_{50}O$ ($M^{+\cdot}$, m/z 426), colour tests[8,14] indicated it to be a triterpene. The melting point agreed with that of friedelin. Its identity as **friedelin (III)** was established by comparison of its ^{1}**H-nmr (Fig.-X, Table-3), ir (Fig.-XI)** and **mass** spectra **(Fig.-XII)** with an authentic sample[11-13] of friedelin.



(III)

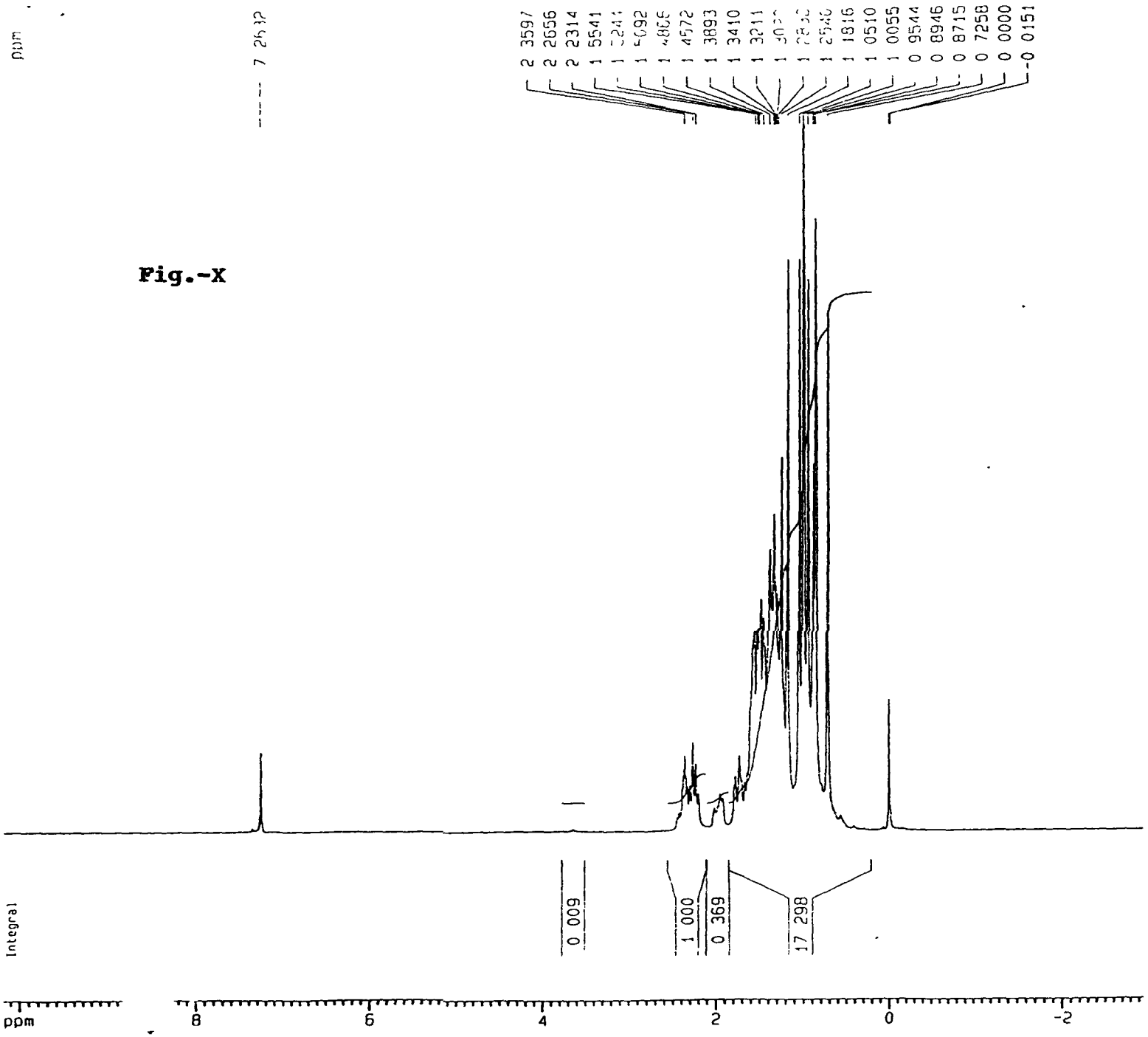
Table-3

^{1}H-NMR spectral data of At-3

Assignment	No. of Protons	Signals
CH_3	(3H, s)	0.72
CH_3	(3H, s)	0.87
CH_3	(3H, s)	0.89
CH_3	(3H, s)	0.92
2 x CH_3	(6H, s)	0.95
CH_3	(3H, s)	1.05
CH_3	(3H, s)	1.18
-CH_2 proton	22 protons	1.25,1.34,1.45,1.52,1.58
C_2-2H C_4-IH	(3H,m)	2.26-2.41 (m)

S=singlet, m=multiplet, spectrum run in $CDCl_3$ at 200 MHz using TMS as internal standard (δ-scale)

Fig.-X



Current Data Parameters

NAME	HM
EXPNO	50
PROCNO	1

F2 - Acquisition Parameters

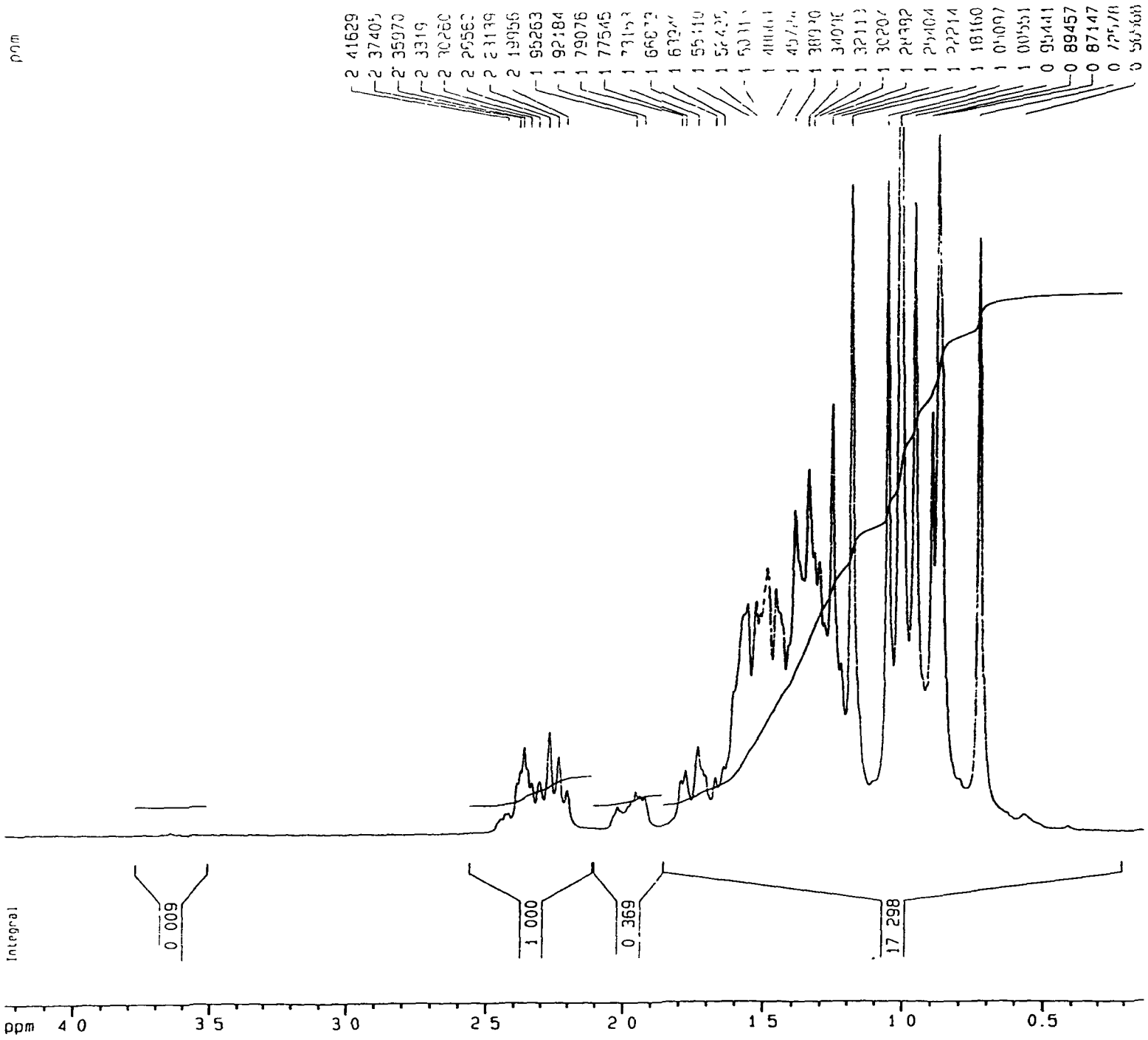
Date_	981007
Time	13.32
INSTRUM	dpx200
PROBHD	5 mm Dual 13
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	32
DS	2
SWH	4111.842 Hz
FIDRES	0.125483 Hz
AQ	3 9846387 sec
RG	362
DW	121.600 usec
DE	6.00 usec
TE	300.0 K
D1	1.00000000 sec
P1	9.00 usec
DE	6.00 usec
SFO1	200.1312359 MHz
NUC1	1H
PL1	-4.00 dB

F2 - Processing parameters

SI	16384
SF	200 1300079 MHz
WDW	EM
SSB	0
LB	0 30 Hz
GB	0
PC	1 00

1D NMR plot parameters

CX	20 00 cm
F1P	10 179 ppm
F1	2037 04 Hz
F2P	-2.948 ppm
F2	-589.97 Hz
PPMCM	0 65633 ppm/cm
HZCM	131 35051 Hz/cm



Current Data Parameters

NAME	[illegible]
EXPNO	50
PROCNO	1

F2 - Acquisition Parameters

Date_	951007
Time	13 32
INSTRUM	cpx200
PROBHD	5 mm Dual 13
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	32
DS	2
SWH	4111.842 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	362
DW	121.600 usec
DE	6.00 usec
TE	300.0 K
D1	1.00000000 sec
P1	9.00 usec
DE	6.00 usec
SFO1	200.1312359 MHz
NUC1	1H
PL1	-4.00 dB

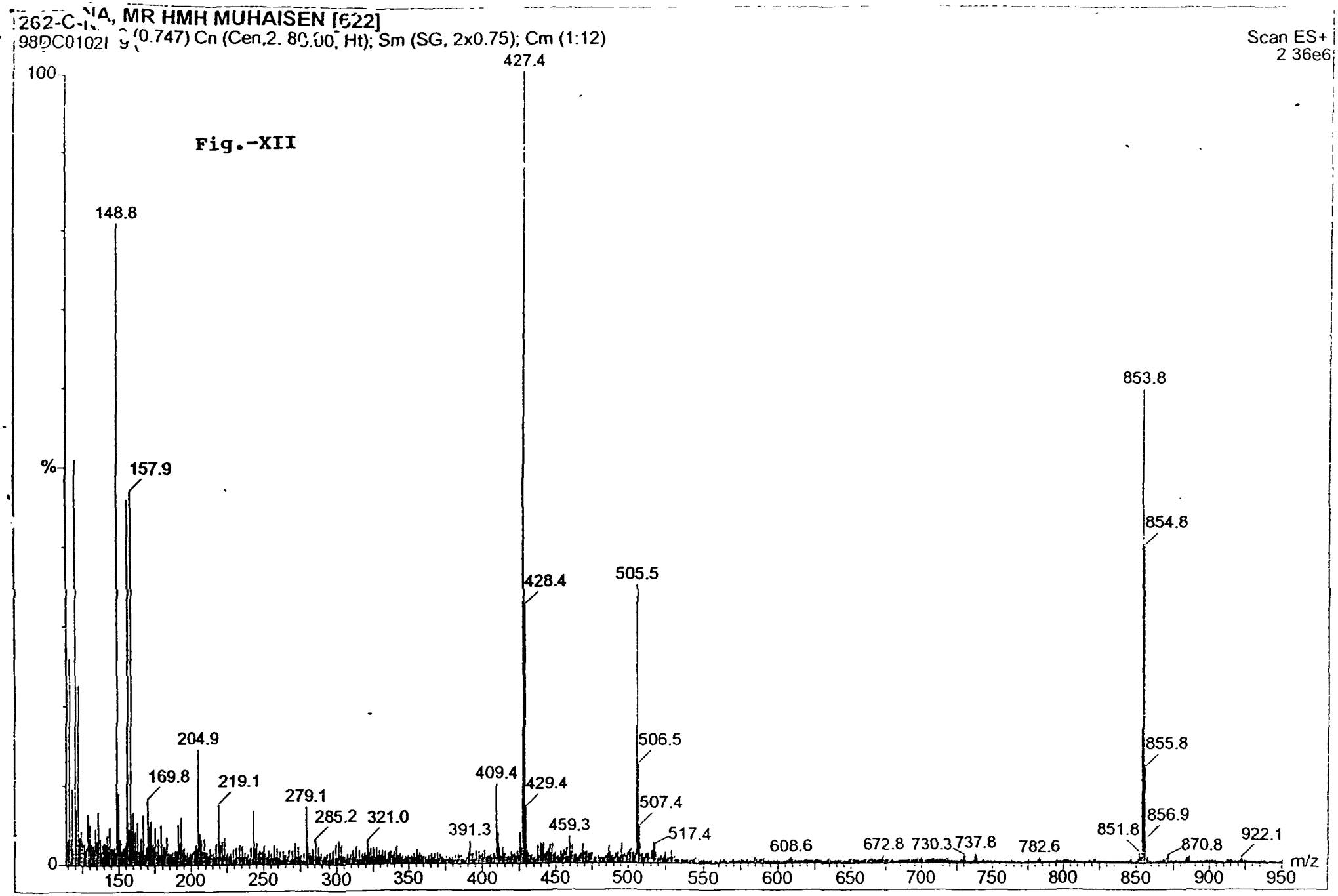
F2 - Processing parameters

SI	16384
SF	200.1300079 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	4.238 ppm
F1	848.20 Hz
F2P	0.121 ppm
F2	24.21 Hz
PPMCM	0.20586 ppm/cm
HZCM	41.19906 Hz/cm

Fig.-XI

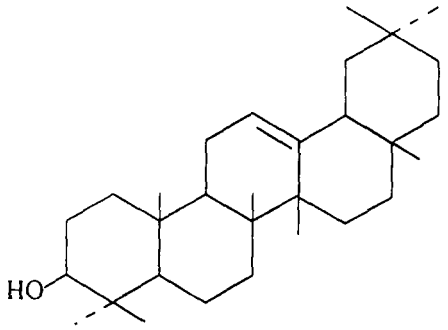

262-C-I... NA, MR HMH MUHAISEN [622]
98DC01021 9 (0.747) Cn (Cen,2. 80.00, Ht); Sm (SG, 2x0.75); Cm (1:12)
Scan ES+
2 36e6
Fig.-XII
100
%
0
427.4
148.8
157.9
853.8
854.8
428.4
505.5
204.9
506.5
855.8
169.8
219.1
409.4
429.4
279.1
507.4
285.2
321.0
391.3
459.3
517.4
608.6
672.8
730.3
737.8
782.6
851.8
856.9
870.8
922.1
m/z
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950

At-4:

It was eluted from the column with Petrol-benzene (2:3) and crystallized with chloroform-methanol as white crystalline solid m.p. 198^{0}C. $[\alpha]^{19}_D$+88.4^0 ($CDCl_3$). It gave positive Leiberman-Burchard test.[14] The **Infrared** spectrum showed the bands at 3360 cm^{-1} (OH), 2960 cm^{-1}, 2880 cm^{-1}, 1650 cm^{-1}, 1465 cm^{-1} (C=C), 1040 and 980 cm^{-1} indicating the presence of (OH) and (olefinic group).

The **mass** spectrum of **At-4** gave molecular ion peak at m/z 426 and analysed for $C_{30}H_{50}O$. Its **^{1}H-nmr** data are given in **(Table-4)**.

From the above data and their direct comparison with an authentic sample, **At-4** was identified as **β-Amyrin**[15] **(IV)**.



(IV)

Table-4

^{1}H-NMR spectral data of At-4

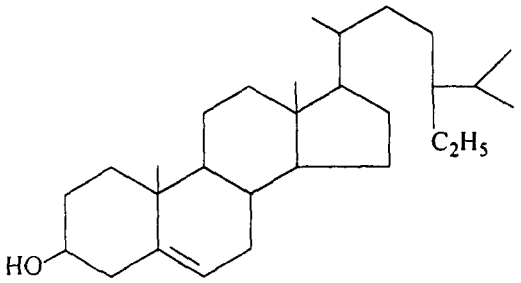
Assignment	No. of Protons	Chemical shift of Protons
8 x CH_3	24	0.78 (s, 3H), 0.83 (s, 3H), 0.88 (s, 6H), 0.95 (s, 3H), 0.98 (s, 3H), 1.0 (s, 3H), 1.14 (s, 3H)
-CH_2 and –CH protons of cyclic system and side chain		1.08, 2.01, 3.01 (dd, J=9 Hz & 7 Hz)
-OH	1	4.88 (s, br)
Olefinic proton	1	5.21 (m)

s= singlet, dd=double doublet, br=broad, m=multiplet, spectrum run in $CDCl_3$ at 100 MHz using TMS as internal standard (δ-scale).

At-5:

The fraction eluted from the column with Petrol-benzene (3:7) mixture gave white crystalline solid m.p. 136-37^{0}C, $[\alpha]_D$ –32.1^0 ($CDCl_3$). **At-5** gave an acetate with acetic anhydride and pyridine m.p. 114-16^{0}C, $[\alpha]_D$ –48.5^0 ($CHCl_3$) and benzoate m.p. 145-46^{0}C. It gave positive Leibermann-Buchard test[14] and responded to the tetranitromethane colour test. The **ir** spectrum showed the presence of gem-dimethyl groups, hydroxyl group and olefinic double bond and showed bands at 3340 cm^{-1} (OH), 1055 cm^{-1}, 840 cm^{-1}, 1460 cm^{-1} (C=C), 1375 cm^{-1} (C-Me_2). The **^{1}H-nmr** data are given in **(Table-5)**. The **mass** spectrum showed the molecular ion peak at m/z 414.

From the above data and direct comparison with an authentic sample[15], **At-5** was characterized as **β-sitosterol (V)**.



(V)

Table-5

^{1}H-NMR spectral data of At-5

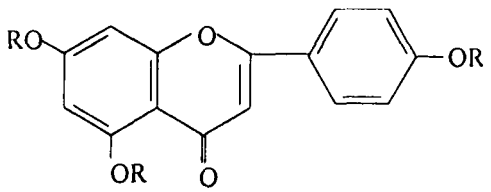
Assignment	Chemical shift of Protons
18-CH_3	0.70 (s, 3H)
28-CH_3	0.80 (d, J=6.8 Hz, 3H)
26, 27-CH_3	0.88 (d, J=6.5 Hz, 6H)
21-CH_3	0.92 (d, J=6.5 Hz, 3H)
19-CH_3	1.02 (s, 3H)
3-ax-H	3.56(m, 1H)
Olefinic proton	5.36 (m, 1H)
-CH_2 and –CH proton of cyclic system and side chain	1.07-2.34

s= singlet, d= doublet, m=multiplet, spectrum run at 90 MHz using TMS as internal standard (δ-scale).

At-6:

It was obtained by elution of column of methanol extract by benzene-ethylacetate (1:1) mixture and crystallized with benzene-acetone as yellow crystals, m.p. 352^{0}C. It gave greenish brown colour with alcoholic $FeCl_3$ and pink colour with zinc and hydrochloric acid, pointing out the presence of flavone nucleus. It was characterized as **apigenin (VI-a)** by comparison with an authentic sample (R_f-value, m.p., m.m.p, co-chromatography), further confirmed by **^{1}H-nmr** spectrum of its acetate (VI-b) **(Table-6)** m.p. 183-84^{0}C.

On the basis of above data the compound **(At-6)** was characterized as **5,7,4'-trihydroxy flavone (Apigenin)[16] (VI-a)** which was further supported by **mass** spectrum which gave a molecular ion peak at m/z 270.



(VI)

(a) R=H
(b) R=Ac

Table-6

^{1}H-NMR spectral data of At-6 acetate

Assignment	No. of Protons	Signals
H-2',6'	2	7.85 (d, J=9 Hz)
H-3',5'	2	7.04 (d, J=9 Hz)
H-3	1	6.60 (s)
H-8	1	6.66 (d, J=2.5 Hz)
H-6	1	6.51(d, J=2.5 Hz)
OAc-5	3	2.42 (s)
OAc-4',7	6	2.35 (s)

s= singlet, d= doublet, spectrum run in $CDCL_3$ at100 MHz using TMS as internal standard (δ-scale)

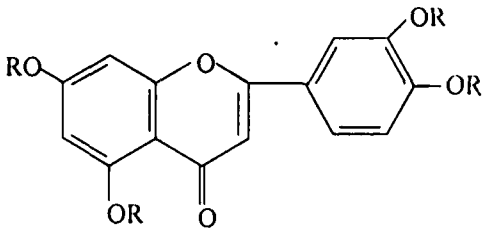
At-7:

It was eluted from the column by benzene-ethyacetate (1:1) mixture as yellow solid which was purified by crystallization from ethylacetate-acetone m.p. >315^{0}C. Elemental analysis agreed to the molecular formula $C_{15}H_{10}O_6$. It responded positively to shinoda's test[17] and gave greenish brown colour with $FeCl_3$. The **uv** spectrum showed λ^{MeOH}_{max} at 258, 265 and 346 nm. Analysis of functional groups revealed the presence of phenolic OH (3400 cm^{-1}), α, β-unsaturated ketonic group >C=O (1640 cm^{-1}) and aromatic nucleus (800 and 840 cm^{-1}). The **uv** spectrum in the presence of diagnostic shift reagents[18] indicated the presence of free hydroxyl groups at 5 and 7 positions and 3',4'-dihydroxyl groups.

Acetylation of **At-7** gave a tetraacetate **(At-7Ac)** (VII-b) m.p. ≈ 200-201^{0}C. The **^{1}H-nmr** spectrum of **At-7Ac (Table-7)** evidenced the presence of four aromatic acetoxyls integrating for 12 protons at δ 2.43 (3H), 2.35 (3H) and 2.33 (6H) assigned to OAc-5, OAc-7 and OAc-3',4' respectively. **^{1}H-NMR** also indicated a

disubstituted ring-B as it showed a typical one proton double doublet at δ 7.75 (J_1=9 Hz and J_2=2.20 Hz, H-6') and a doublet of one proton δ 7.80 (J=2.20 Hz, H-2'). This could be attributed to 3',4'- substitution of ring-B. Another ortho coupled doublet integrating for one proton at δ 7.25 (J=9 Hz) was ascribed to H-5'. A pair of meta-coupled doublets centered at δ 6.45 (J=2.5 Hz) and 6.95 (J=2.5 Hz) were assigned to C-6 and C-8 protons of ring-A, while C-3 proton of pyrone ring resonated as a sharp singlet at δ 6.59. The **mass** spectrum showed a molecular ion peak at m/z 286 corresponding to the structure (VII-a). The fragment ions at m/z 134 fully supported a ring-B with two hydroxyl groups. Fragment at m/z 153 was consistent with the ring-A having two hydroxyl groups.

On the basis of these results **At-7** was characterized as **5,7,3',4' tetrahydroxy flavone (Luteolin)[16] (VII-a).**



(VIII)

(a) R=H
(b) R=Ac

Table-7

^{1}H-NMR spectral data of At-7 acetate

Assignment	No. of Protons	Signals
H-6	1	6.45 (d, J=2.5 Hz)
H-8	1	6.95 (d, J=2.5 Hz)
H-3	1	6.59 (s)
H-5'	1	7.25 (d, J=9 Hz)
H-2'	1	7.80 (d, J=2.20 Hz)
H-6'	1	7.75 (dd, J_1=9 Hz & J_2=2.20 Hz)
OAc-5	3	2.43 (s)
OAc-7	3	2.35 (s)
OAc-3',4'	6	2.33 (s)

s= singlet, d= doublet, dd= double doublet, spectrum run in $CDCl_3$ at 270 MHz using TMS as internal standard (δ-scale).

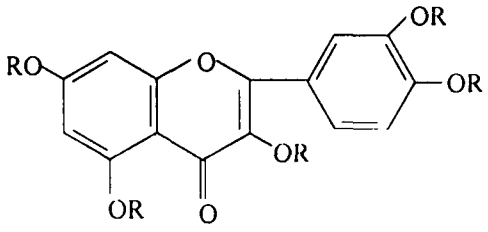
At-8:

It was crystallized with methanol, as yellow crystals, m.p. 311-12^{0}C and was characterized as **quercetin** by co-chromatography and mixed melting point with an authentic sample. On acetylation with acetic anhydride and pyridine, it gave an acetate m.p. 194-95^{0}C. The identity of the compound as quercetin was further confirmed by spectral studies (**uv** and **^{1}H-nmr** spectra) **(Table-8 and Table-9)**.

The **^{1}H-nmr** spectrum of its acetate (VIII-b) showed the signals due to five acetoxyls at δ 2.35-2.40. A pair of meta-coupled doublet at δ 6.87 (J=2.5 Hz) and

δ 6.65 (J=2.5 Hz) was assigned to H-8 and H-6 protons of ring-A respectively. The ring-B protons showed an ABX pattern, two doublet at δ 7.74 (J=2.5 Hz) for H-2' and δ 6.92 (J=8.5 Hz) for H-5' and a quartet at δ 7.63 (J=2.5 Hz and 8.5 Hz) for H-6'.

In the light of above results compound **(At-8)** was assigned the structure as **3, 5,7, 3',4'-pentahydroxy flavone (quercetin)[19] (VIII-a).**



(VIII)

(a) R=H
(b) R=Ac

Table-8

UV data of At-8 and quercetin

Reagent	At-8	Quercetin
MeOH	256,270 sh, 301 sh, 372	255, 269 sh, 301 sh, 370
NaOMe	247 sh. 321 (Dec)	247 sh, 321 (Dec)
$AlCl_3$	274, 304 sh, 334, 458	272, 304 sh, 333, 458
$AlCl_3$/HCl	264, 358, 427	265, 301 sh, 359, 428
NaOAc	257 sh, 274, 329, 390	257, 274, 390 (Dec)
NaOAc/H_3BO_3	264, 303 sh, 389	261, 301 sh, 388

Table-9

^{1}H-NMR spectral data of At-8

Assignment	No. of Protons	Signals
H-2'	1	7.74 (d, J=2.5 Hz)
H-6'	1	7.63 (q, J_1=2.5 Hz, J_2=8.5 Hz)
H-5'	1	6.92 (d, J=8.5 Hz)
H-8	1	6.87 (d, J=2.5 Hz)
H-6	1	6.65 (d, J=2.5 Hz)
5 x OAc	15	2.35 (m), 2.40 (s)

s= singlet, d= doublet, q=quartet, m=multiplet, spectrum run in $CDCl_3$ at 100 MHz using TMS as internal standard (δ-scale).

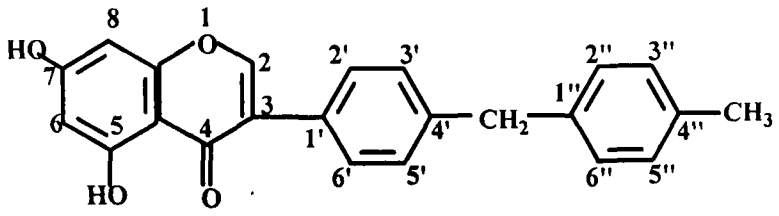
At-9:

It was eluted from the column by ethylacetate and was crystallized with methanol as pale yellow granular crystals m.p168^{0}C analysed for $C_{23}H_{18}O_4$. It gave a greenish brown colour with alcoholic ferric chloride, and a pink colour with sodium amalgum / HCl and yellow colour with conc. H_2SO_4[20]. The colour test and **uv** absorption, λ_{max}262 and inflection at 339 nm indicated isoflavone nucleus, further supported by a singlet in its **^{1}H-nmr** spectrum at δ 7.86 ascribed to H-2 proton of isoflavone. A red shift of 10 nm with $AlCl_3$ and 11 nm with NaOAc showed the presence of hydroxyl group at 5 and 7 positions which was further confirmed by the appearance of the singlets at δ 12.46 and δ 9.27 in the **^{1}H-nmr** spectrum **(Fig.-XIII, Table-10)**.

The **^{1}H-nmr** spectrum displayed a singlet integrating for 3 protons at δ 2.50 corresponding to methyl group and a pair of meta-coupled doublets of one proton each at δ 6.17 (J=2.5 Hz) and 6.40 (J=2.5 Hz), attributed to H-6 and H-8 protons of ring-A respectively. Another pair of ortho coupled doublets of four protons each at δ 6.89 (J=9 Hz) and 7.56 (J=9 Hz) were assigned to H-3',5',3",5" and H-2', 6',2"',6" respectively. A solitory one proton singlet at δ 7.86 was ascribed to H-2 proton of isoflavone. The CH_2 protons appeared at δ 2.59.

The assigned structure was further supported by the **mass** spectrum **(Fig.-XIV Scheme-III)** which showed the molecular ion peak at m/z 358. The RDA fragments appeared at m/z 152, 206 and the base peak was observed at m/z 91 corresponded to tropolium ion.

On the basis of above studies it was characterized as the novel isoflavone named as **5,7-dihydroxy-4'-p-methyl benzyl isoflavone (IX).**



(IX)

Table-10

^{1}H-NMR spectral data of At-9

Assignment	No. of Protons	Signals
CH_3	(3H, s)	2.50
CH_2	(2H, s)	2.59
H-6	(1H, d, J=2.5 Hz)	6.17
H-8	(1H, d, J=2.5 Hz)	6.40
H-3',5',3",5"	(4H, d, J=9 Hz & 2.5 Hz)	6.89
H-2',6',2"',6"	(4H, d, J=9 Hz & 2.5 Hz)	7.56
H-2	(1H, s)	7.86
CH_2	(2H, s)	2.59
7-OH	(1H, brs)	9.27
5-OH	(1H, s)	12.46

s= singlet, brs= broad singlet, d= doublet, spectrum run in DMSO-d_6 at 300 MHz using TMS as internal standard (δ-scale).

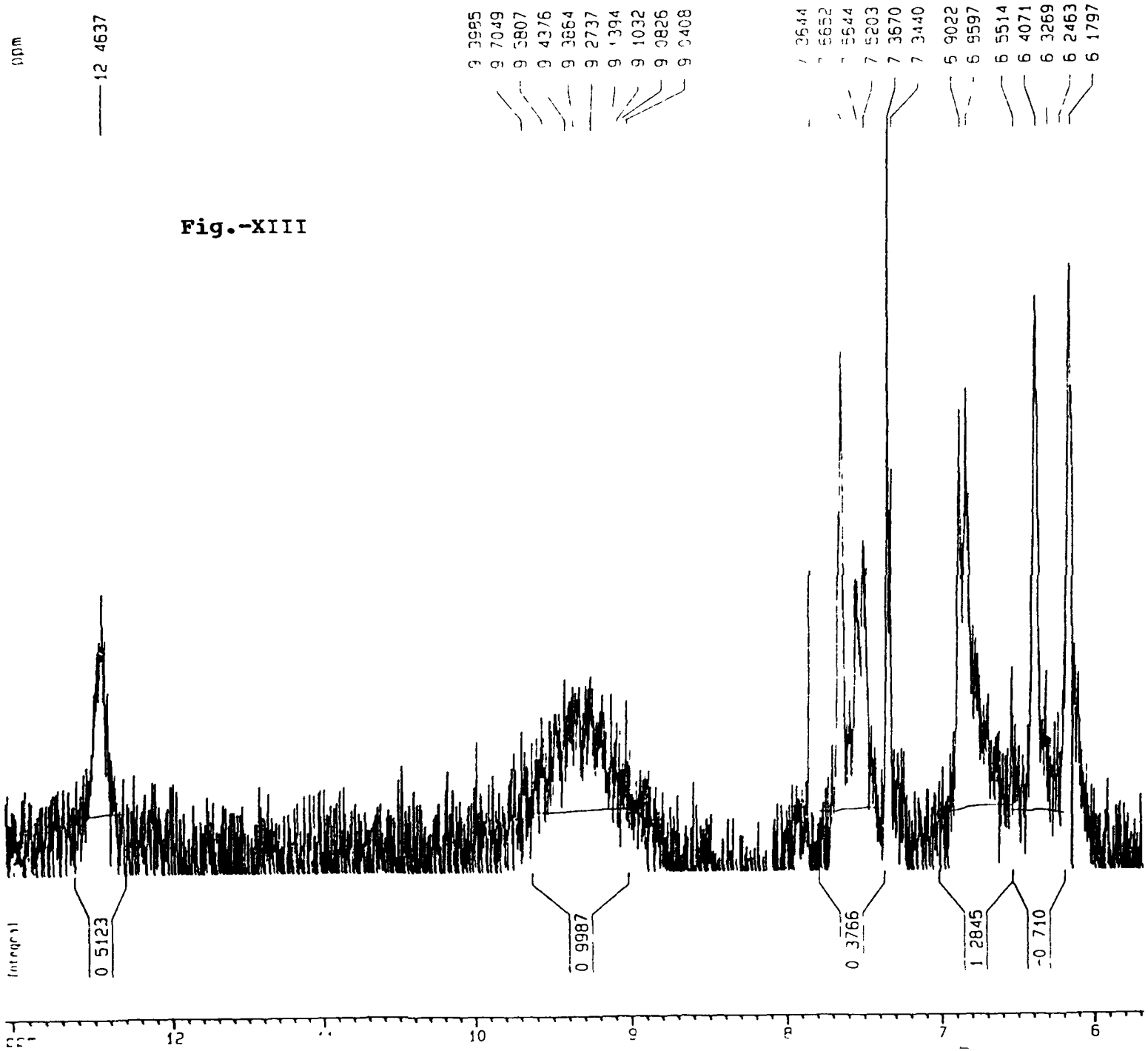


Fig.-XIII

Current Data Parameters

NAME	Feb05
EXPNO	180
PROCNO	1

F2 - Acquisition Parameters

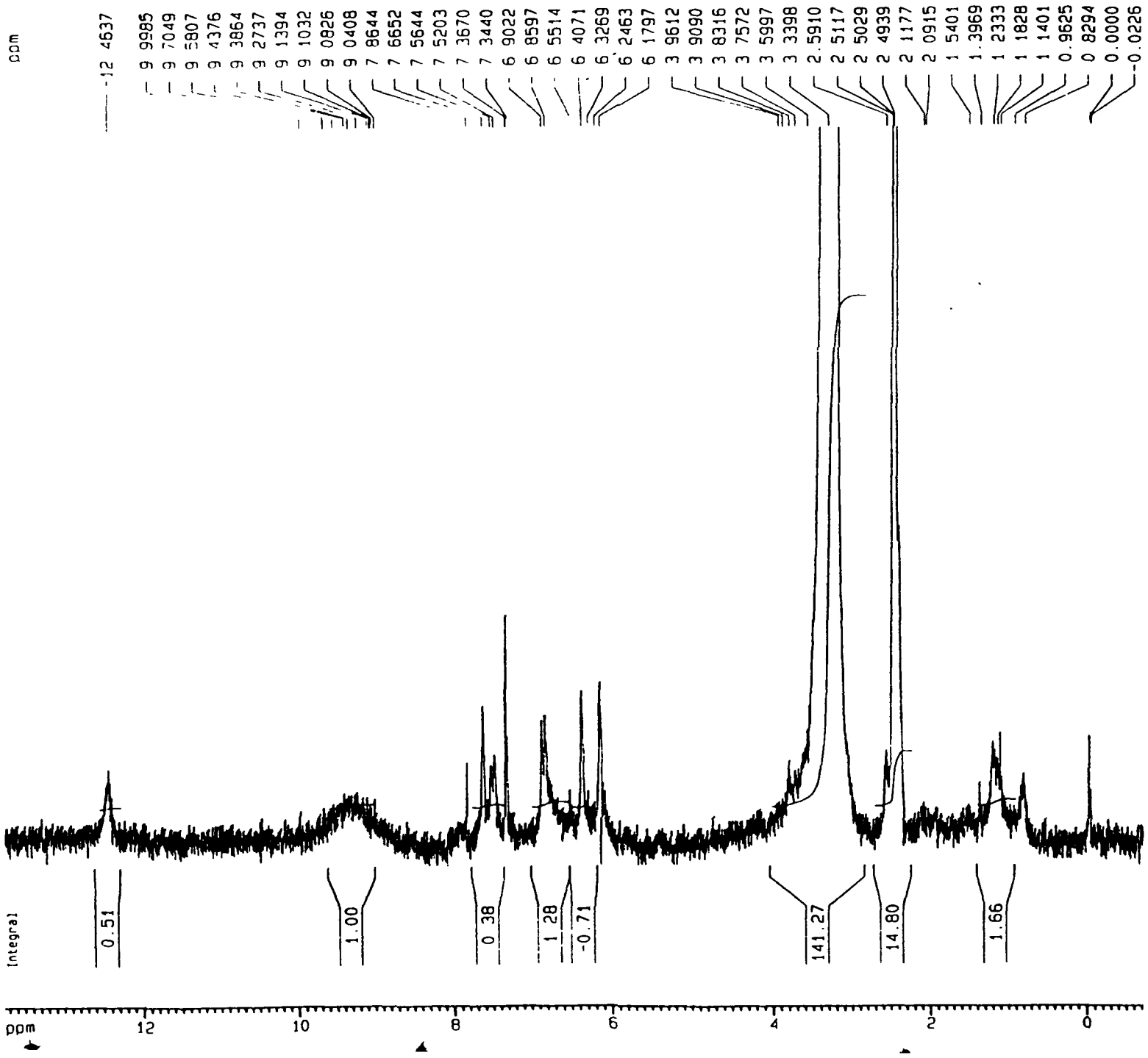
Date_	500000
Time	16 29
INSTRUM	dpx200
PROBHD	5 mm Dual 13
PULPROG	zg30
TD	32768
SOLVENT	DMSO
NS	16
DS	2
SWH	4111 842 Hz
FIDRES	0 125483 Hz
AQ	3 9846387 sec
RG	812 7
DW	121 600 usec
DE	6 00 usec
TE	300 0 K
D1	1 00000000 sec
P1	9 00 usec
DE	6 00 usec
SFO1	200 1312359 MHz
NUC1	1H
PL1	-4 00 dB

F2 - Processing parameters

SI	16384
SF	200 1300051 MHz
WDW	EM
SSB	0
LB	0 30 Hz
GB	0
PC	1 00

1D NMR plot parameters

CX	20 00 cm
F1P	13 051 ppm
F1	2611 82 Hz
F2P	5 681 ppm
F2	1136 93 Hz
PPMCM	0 36849 ppm/cm
HZCM	73 74499 Hz/cm



Current Data Parameters

NAME	Feb05
EXPNO	180
PROCNO	1

F2 - Acquisition Parameters

Date_	500000
Time	16 29
INSTRUM	dpx200
PROBHD	5 mm Dual 13
PULPROG	zg30
TD	32768
SOLVENT	DMSO
NS	16
DS	2
SWH	4111 842 Hz
FIDRES	0.125483 Hz
AQ	3 9846387 sec
RG	812.7
DW	121 600 usec
DE	6.00 usec
TE	300.0 K
D1	1 00000000 sec
P1	9.00 usec
DE	6.00 usec
SFO1	200.1312359 MHz
NUC1	1H
PL1	-4.00 dB

F2 - Processing parameters

SI	16384
SF	200 1300051 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1 00

1D NMR plot parameters

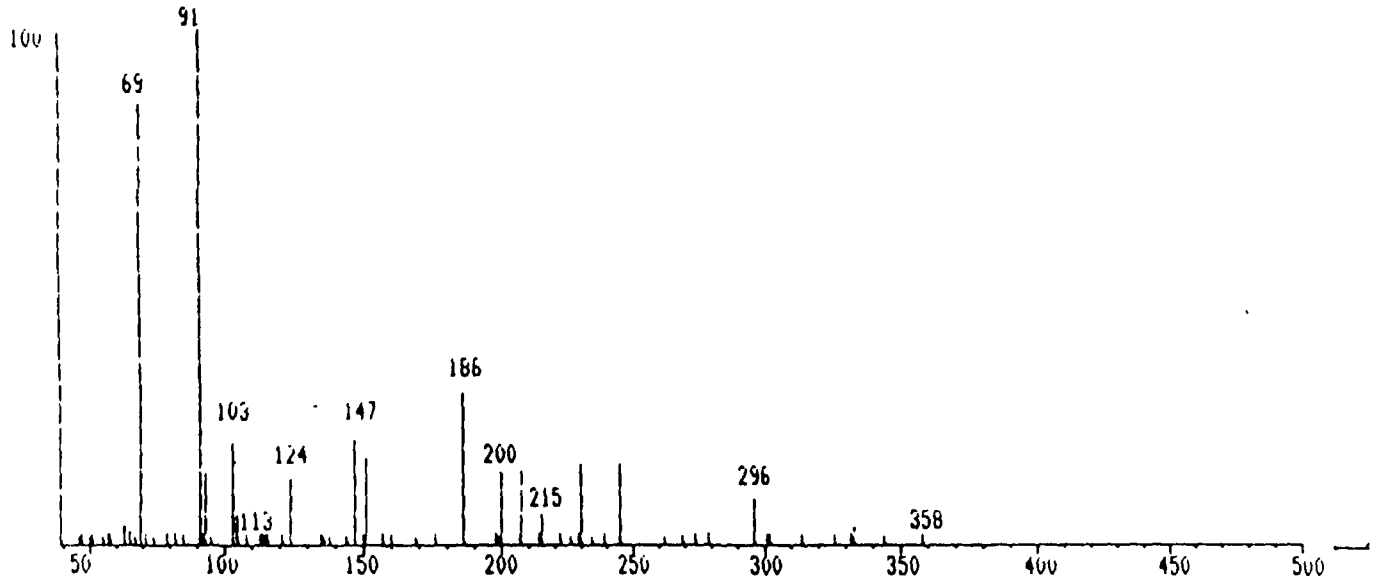
CX	20.00 cm
F1P	13.765 ppm
F1	2754.85 Hz
F2P	-0.706 ppm
F2	-141.32 Hz
PPMCM	0 72357 ppm/cm
HZCM	144.80835 Hz/cm

अनु० सं०
R. I.

CENTRAL DRUG RESEARCH INSTITUTE
12-27-1999

DC2711X.LRP DR H MUHAISEN/AMU,ALIGARH/1
Date run : 12-27-1999 Operator : PRAKASH/A.SONI/SUNIL

Scan : 23 RT= 3:50 No.ions= 66 Base= 13.8%F TIC= 23277



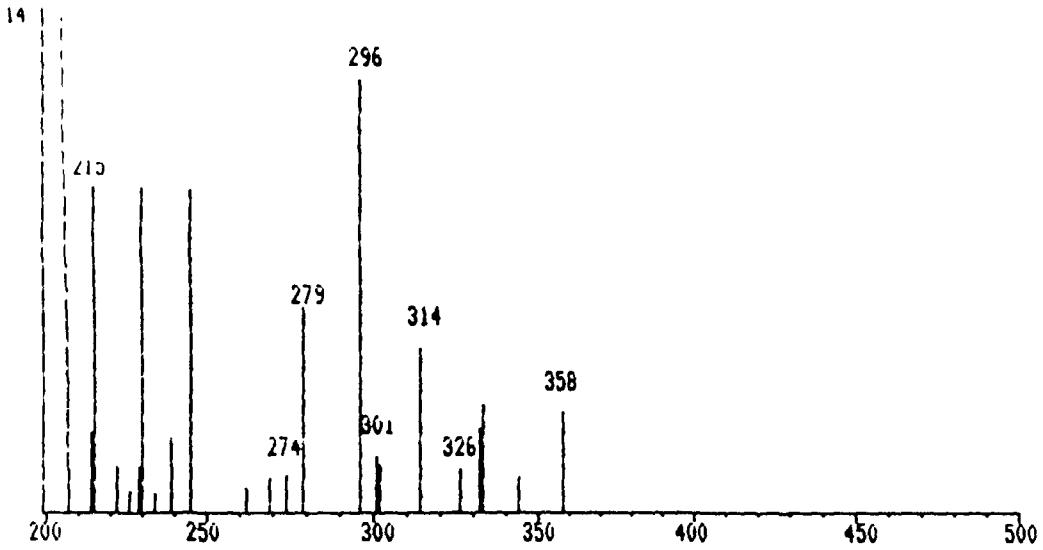
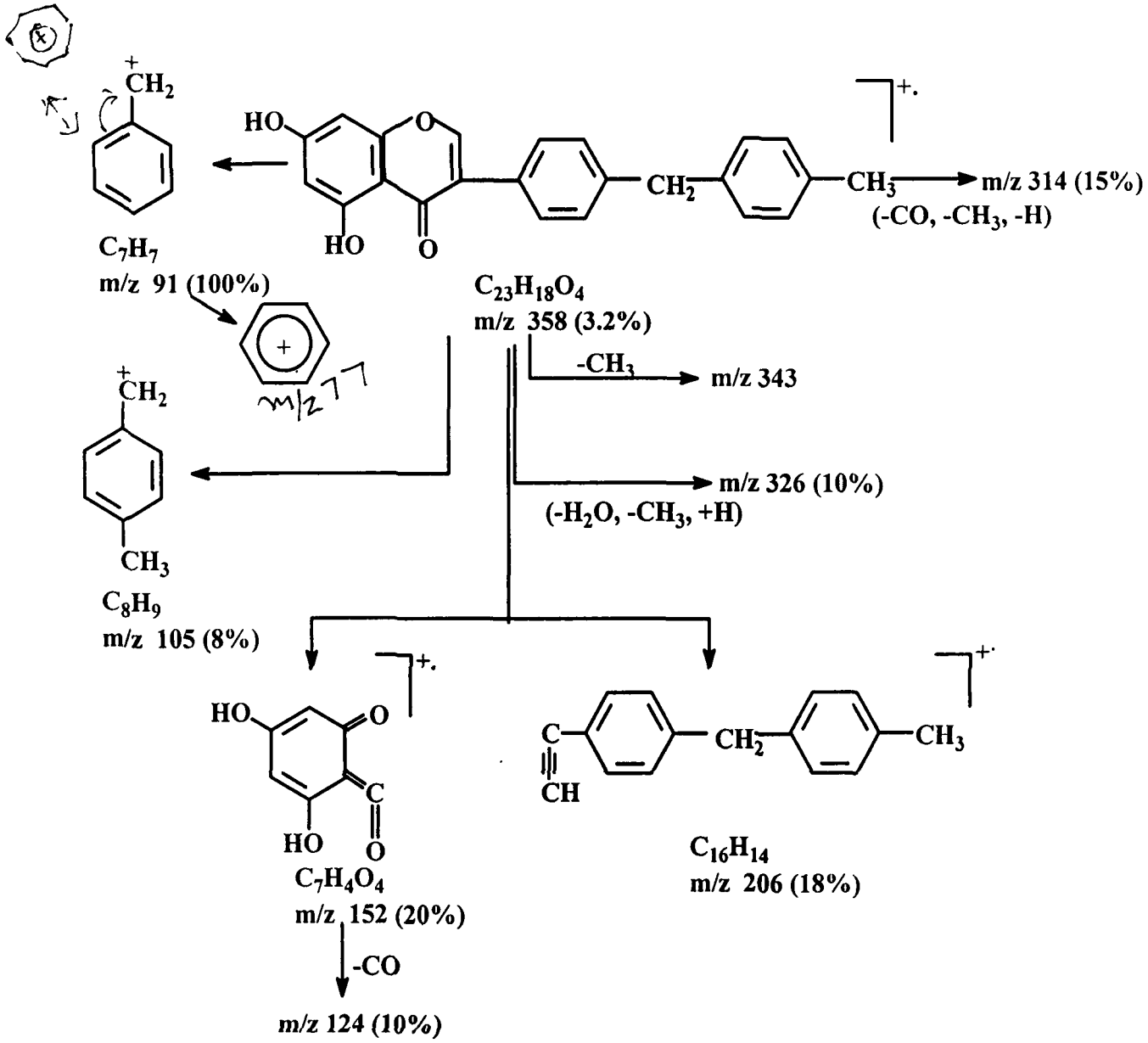


Fig.-XIV


HO
O
HO
O
CH2
CH3
m/z 314 (15%)
(-CO, -CH3, -H)
C7H7
m/z 91 (100%)
C23H18O4
m/z 358 (3.2%)
-CH3
m/z 343
m/z 326 (10%)
(-H2O, -CH3, +H)
CH3
C8H9
m/z 105 (8%)
HO
O
HO
O
C7H4O4
m/z 152 (20%)
-CO
m/z 124 (10%)
C
CH
CH2
CH3
C16H14
m/z 206 (18%)

(Scheme-III)

At-10:

At-10 was eluted from the column by ethylacetate-methanol (9:1). On crystallization with methanol-chloroform, it gave yellow crystals m.p. 263-64^{0}C. The molecular ion peak at m/z 432 and the elemental analysis agreed with the molecular formula as $C_{21}H_{20}O_{10}$. A dark reddish colour with magnesium and hydrochloric acid and a red colour on treatment with sodium amalgam, followed by acidification suggested a flavone nucleus for the compound.

The **At-10** gave a positive Molish test and a dark brown colour with ferric chloride. The analysis of functional groups revealed the presence of α,β-unsaturated >C=O (1650), phenolic OH (3420), and a complex aromatic substitution, besides a strong band at 2950 cm^{-1}. The uv spectrum showed λ^{MeOH}_{max} at 268 and 335 nm. The red shift of 10 nm with NaOAc in band-II, 11 nm with $AlCl_3$ and 38 nm with NaOMe in band I (without decrease in intensity) indicated the presence of 5,7 and 4' hydroxyls groups.

Prolonged heating (5 hours) of the glycoside with 0.4 M.HCl failed to hydrolyse the glycoside, suggestive of a C-glycosyl nature of the compound. This was further supported by two bands at 1010 and 1038 cm^{-1} in its **ir** spectrum.[21] The compound **(At-10)** was oxidised with $FeCl_3$, the sugar obtained was identified as glucose (by m.p., co-chromatography and gc of trimethylsilyl derivative). Pyranose structure of glucose was confirmed by periodate oxidation of methyl ether of the glucoside.

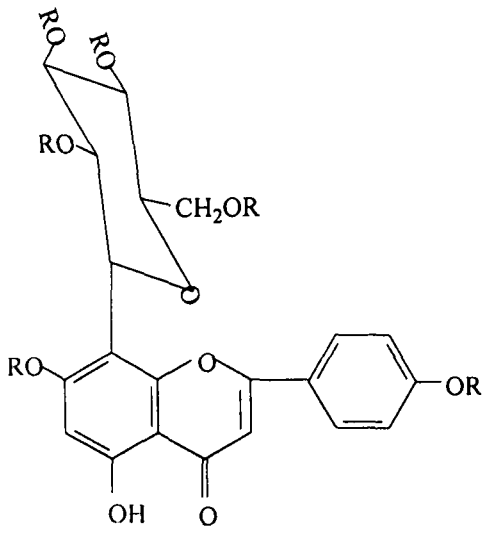
On boiling the glucoside **(At-10)** with hydroiodic acid in phenol, the sugar moiety was decomposed. The resulting product was identified as apigenin (At-10ag) by comparison of m.p. and spectral data **(uv, ir, 1H-nmr** and **mass)** with those of an authentic samples[22]. The **uv** spectrum of the aglycone was found to be identical with that of glucoside. The acetate of the glucoside, prepared by

heating it with acetic anhydride and pyridine gave white crystals (At-10Ac), m.p. 154-156°C analysed for $C_{33}H_{32}O_{16}$.

The **^{1}H-nmr** spectrum **(Table-11)** exhibited a pair of ortho-coupled doublets integrating for two protons each at δ 7.40 (J=9 Hz) and δ 8.10 (J=9 Hz) corresponded to H-3',5' and H-2',6'. Two independent singlets of one proton each at δ 6.8 and δ 6.91 were assigned to H-3 and H-6 respectively. The anomeric proton H-1" (glu) appeared as a doublet at δ 4.64 (J=10 Hz) showing trans diaxial relationship with H-2", while the sugar protons appeared in the range of δ 3.60-5.70. Three singlets at δ 2.32, 2.43 and 2.51 integrating for three protons each were attributed to three aromatic acetoxyls at 4,7 and 5 positions. The four aliphatic acetoxyl appeared as a multiplet at δ 1.72-2.02. The presence of the signals at δ 1.72 for 2"-OAc and 2.02 for 6"-OAc indicatd the presence of sugar moiety at C-8[23]. This was supported by the negative Gibb's test. The location of the sugar at C-8 was further confirmed by the mass fragmentation, as the characteristic fragments at m/z 312 (aglycone attached to CH_2-CHO) was observed[24].

The other fragments observed were at m/z 283 [aglycone attached with CH_2] and 354 [$M^{+\cdot}$-C-glucosyl + H^+]. The fragment ion at m/z 270 seemed to be formed by the loss of 2 x CH_2=C=O groups from m/z 354. The RDA fragments representing ring-A and ring-B were observed at m/z 194 $[A_1]^+$ and at m/z 118 $[B_1]^+$ respectively.

On the basis of the above results, the compound **(At-10)** was characterized as **vitexin (X)**.



(X)

(a) R=H
(b) R=Ac

Table-11

^{1}H-NMR spectral data of At-10 Ac

Assignments	No. of Protons	Signals
H-3	1	6.8 (s)
H-6	1	6.91 (s)
H-3',5'	2	7.4 (d, J=9 Hz)
H-2',6'	2	8.1 (d, J=9 Hz)
H-1"	1	4.64 (d, J=10 Hz)
H-1",2",3",4",5",6"	7	3.65-5.70 (m)
Aromatic acetoxyls:		
OAc	3	2.32 (s)
OAc	3	2.43 (s)
OAc	3	2.51 (s)
Aliphatic acetoxyls	12	1.72-2.02 (m)

s = singlet, m = multiplet, d = doublet, spectrum run in CDCl3 at 300 MHz., using TMSi as internal standard (δ-scale).

At-11:

Elution of the column with ethylacetate-methanol (8:2-7:3) followed by crystallization with methanol-chloroform afforded pale yellow crystals (100 mg) m.p. >280^{0}C. The elemental analysis agreed with the molecular formula $C_{21}H_{22}O_9$. It gave red colour with conc. H_2SO_4 and orange to red colour with aqueous NaOH suggesting it to be a chalcone.[25,26]. Positive Molish test indicated it to be a chalcone glycoside. The **ir** spectrum showed the characteristic bands at 2965 (br, chelated OH), 1684 (C=O) and 1462 (C=C) cm^{-1}. **UV** spectrum showed the absorptions at 365 nm (Band-I) and at 245 nm (Band-II). A red shift of 71 nm with $AlCl_3$ / HCl in Band-I showed the presence of chelated-hydroxyl group[27]. **At-11** gave greenish brown colour with alcoholic $FeCl_3$, indicating the presence of 2' and 6' hydroxyl groups. The placement of hydroxyl groups at 2' and 6' positions was justified by its **^{1}H-nmr** spectrum **(Fig.-XV, Table-12)** which showed two exchangeable hydroxyl groups with D_2O by the off field signals at δ 13.6 (2'-OH) and at δ 12.85 (6'-OH).

Acetylation of the glycoside **(XI-a)** with acetic anhydride and pyridine afforded an acetate **(XI-b)** m.p 178°C. Its **^{1}H-nmr** spectrum indicated it to be an hexaacetate derivative as it exhibited the presence of six acetoxyl groups (two aromatic and four aliphatic). A six protons singlet at δ 2.52 was ascribed to the aromatic acetoxyl at 2' and 6'-position while the aliphatic acetoxyls integrating for twelve protons appeared as a multiplet in the range of δ 1.78 to 2.05. A pair of meta coupled doublets at δ 6.24 (J=2.0 Hz) and δ 6.26 (J=2.0 Hz) were assigned to 3' and 5' protons of ring-A respectively. The ring-B protons (2,3,4,5,6) appeared as multiplet in the range of δ 6.77-6.85. A pair of doublets at δ 6.97 (J=15 Hz) and 7.85 (J=15 Hz) were accounted for α and β-protons of chalcone. The sugar protons appeared as multiplet in the range of 3.5-4.32 and 5.22-5.50. While the anomeric proton of glucose (H-1") was centered as doublet at δ 4.1 (J=9 Hz). The coupling constant supporting the β-linkage of glucose.

At-11 on hydrolysis with 6% HCl yielded a sugar and an aglycone. The sugar was identified as glucose by R_f value and co-chromagraphy with an authentic sample of glucose.

The ultra-violet and infrared spectra of the aglycone and its derivatives showed that it contained a conjugated carbonyl group and three phenolic hydroxyl groups. It gave greenish brown colour with ferric chloride. The hydroxyl groups were placed at 2' and 6' positions of the chalcone as discussed above. The remaining hydroxyl group was placed at 4'-position since it gave a red colour with vanillin-HCl reagent[28] and a red shift of 62 nm in band I with NaOMe in its **uv** spectrum (absent in glycoside). Thus all the three hydroxyl groups were placed in ring-A with no substitution in ring-B (alkaline fusion of the aglycone gave benzoic acid). The presence of a free 4'-OH group in the aglycone which was not found in the glycoside indicated the sugar linkage in the chalcone at 4'-position.

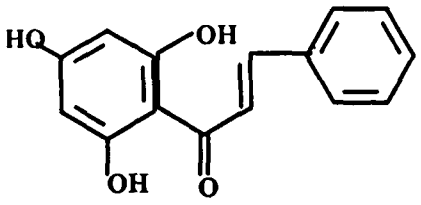
The **^{1}H-nmr** of the aglycone exhibited a pair of meta-coupled doublets at δ 6.20 (J=2.0 Hz) and δ 6.23 (J=2.0 Hz) for H-3' and H-5' protons respectively. The ring-B protons (H-2',3',4',5',6') appeared in the range of δ 6.75-6.82. The remaining α,β protons of the chalcone resonated as a doublet at δ 6.95 (J=15 Hz, H-α) and δ 7.81 (J=15 Hz, H-β). Therefore, the aglycone was characterized as **2',4',6'-trihydroxychalcone**[29] **(XI)**.

The quantitative estimation of the sugar by somogyi's copper-micro method[30] showed the presence of 1 mole of glucose per mole of the aglycone.

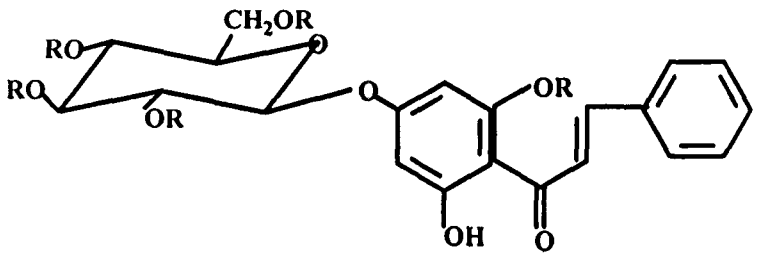
The **mass** spectrum **(Fig.-XVI, Scheme-IV)** of the glycoside (XI-a) was in full agreement with the assigned structure of the glycoside. It showed the presence of glucose at m/z 180 and an aglycone at m/z 256. The retro-Diels-Alder fragmentation pattern was observed by peaks at m/z 152, 131,126 and 104, supporting the presence of three hydroxyl groups in ring-A and no-hydroxyl group in ring-B. It was further confirmed by the degredation[31] of the aglycone with 50%

KOH which gave phloroglucinol and cinnamic acid which were identified by co-TLC with authentic samples.

On the basis of the above results the compound (**At-11**) was thus characterized as **2',6'-dihydroxychalcone-4'-O-glucoside (XI-a)** which is being reported for the first time.



(XI)



(XI-a) R=H
(XI-b) R=Ac

Table-12

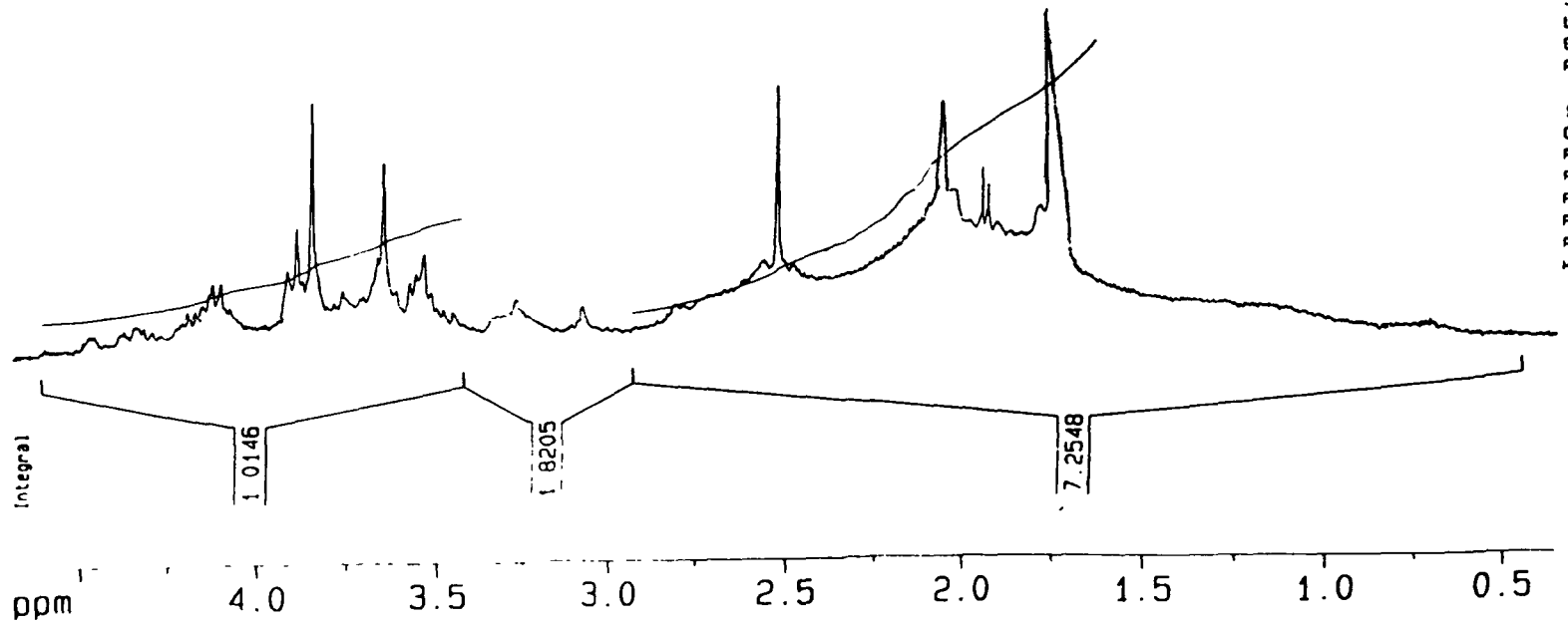
^{1}H-NMR spectral data of At-11

Assignment	No. of Protons	Signals
OAc-2',6'	6	2.52 (s)
H-3'	1	6.24 (d, J=2.0 Hz)
H-5'	1	6.26 (d, J=2.0 Hz)
H-2,3,4,5,6	5	6.77-6.85 (m)
H-α	1	6.97 (d, J=15 Hz)
H-β	1	7.85 (d, J=15 Hz)
Sugar protons:		
H-1" (anomeric)	1	4.1 (d, J=9 Hz)
H-1",2",3",4"	4	3.5-4.32 (m)
H-5",6"	3	5.22-5.50 (m)
Sugar acetoxyls:		
H-2",3",4",6"	12	1.78-2.05 (m)

s= singlet, d= doublet, m=multiplet, spectrum run in DMSO at 300 MHz using TMS as internal standard (δ-scale).

ppm
0
2
4
6
8
1 8205
1 0146
2330
3123
7 2548
0 003
-1 784
-1 834
-1 866
-1 900
-1 928
-1 945
-2 053
2 524
3 537
3 648
3 886
4 866
5 244
5 267
5 304
5 332

Fig.-XV



Current Data Parameters

NAME	
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20010301
Time	9.41
INSTRUM	DRX300
PROBHD	5 mm Multinu
PULPROG	zgpr
TD	32768
SOLVENT	MeOH
NS	16
DS	0
SWH	5995.205 Hz
FIDRES	0.182959 Hz
AQ	2.7329006 sec
RG	362
DW	83.400 usec
DE	6.00 usec
TE	297.0 K
D1	1.00000000 sec
d12	0.00002000 sec
d13	0.00000300 sec

********** CHANNEL f1 **********

NUC1	1H
P1	6.88 usec
PL1	-3.00 dB
PL9	55.00 dB
SF01	300.1314698 MHz

F2 - Processing parameters

SI	16384
SF	300.1300050 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	4.690 ppm
F1	1407.64 Hz
F2P	0.349 ppm
F2	104.75 Hz
PPMCM	0.21705 ppm/cm
HZCM	65.14454 Hz/cm

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_              20010301
Time                   9 41
INSTRUM             DRX300
PROBHD     5 mm Multinu
PULPROG            zgpr
TD                    32768
SOLVENT                MeOH
NS                       16
DS                        0
SWH                5995 205 Hz
FIDRES             0 182959 Hz
AQ                 2 7329006 sec
RG                      362
DW                 83 400 usec
DE                 6 00 usec
TE                 297 0 K
D1                 1 00000000 sec
D12                0 00000200 sec
D13                0 00000300 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                     6 88 usec
PL1                    -3 00 dB
PL9                    55 00 dB
SF01           300 1314698 MHz

F2 - Processing parameters
SI                     16384
SF             300 1300050 MHz
WDW                    EM
SSB                       0
LB                     0 30 Hz
GB                        0
PC                     1 00

1D NMR plot parameters
CX                    20 00 cm
F1P                   9 077 ppm
F1                 2724 42 Hz
F2P                   4 674 ppm
F2                 1402 70 Hz
PPMCM              0 22019 ppm/cm
HZCM               66 09801 Hz/cm
```

o औ० अनु० स०
. D. R. I.

CENTRAL DRUG RESEARCH INSTITUTE
01-15-2001

JN1513X.LRP MR H MUHAISEN/ALIGARH #2795
Date run : 01-15-2001 Operator : PRAKASH/A.SONI/SUNIL

Scan : 15 RT= 1:42 No.ions= 307 Base= 23.4%F TIC=189190

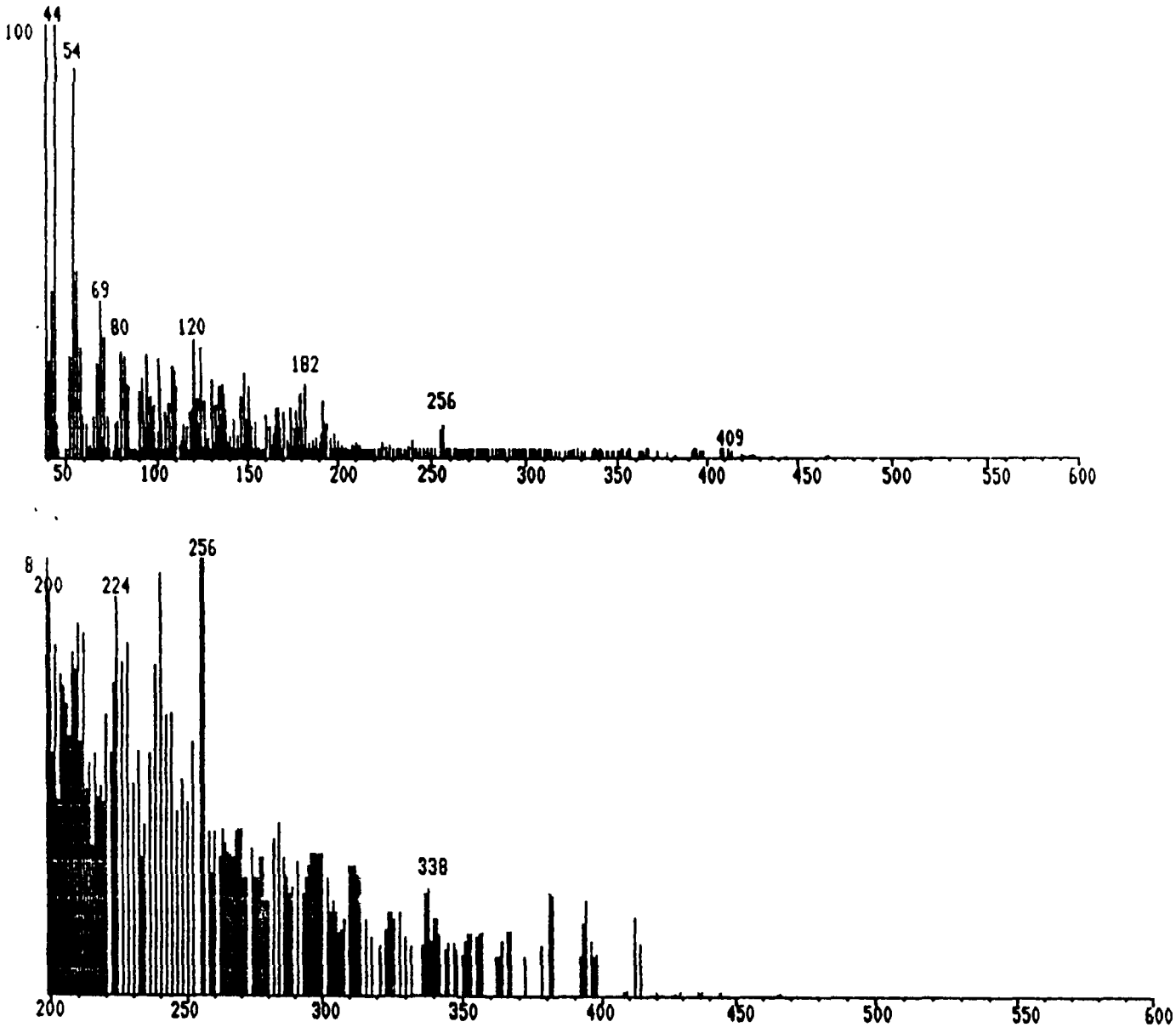
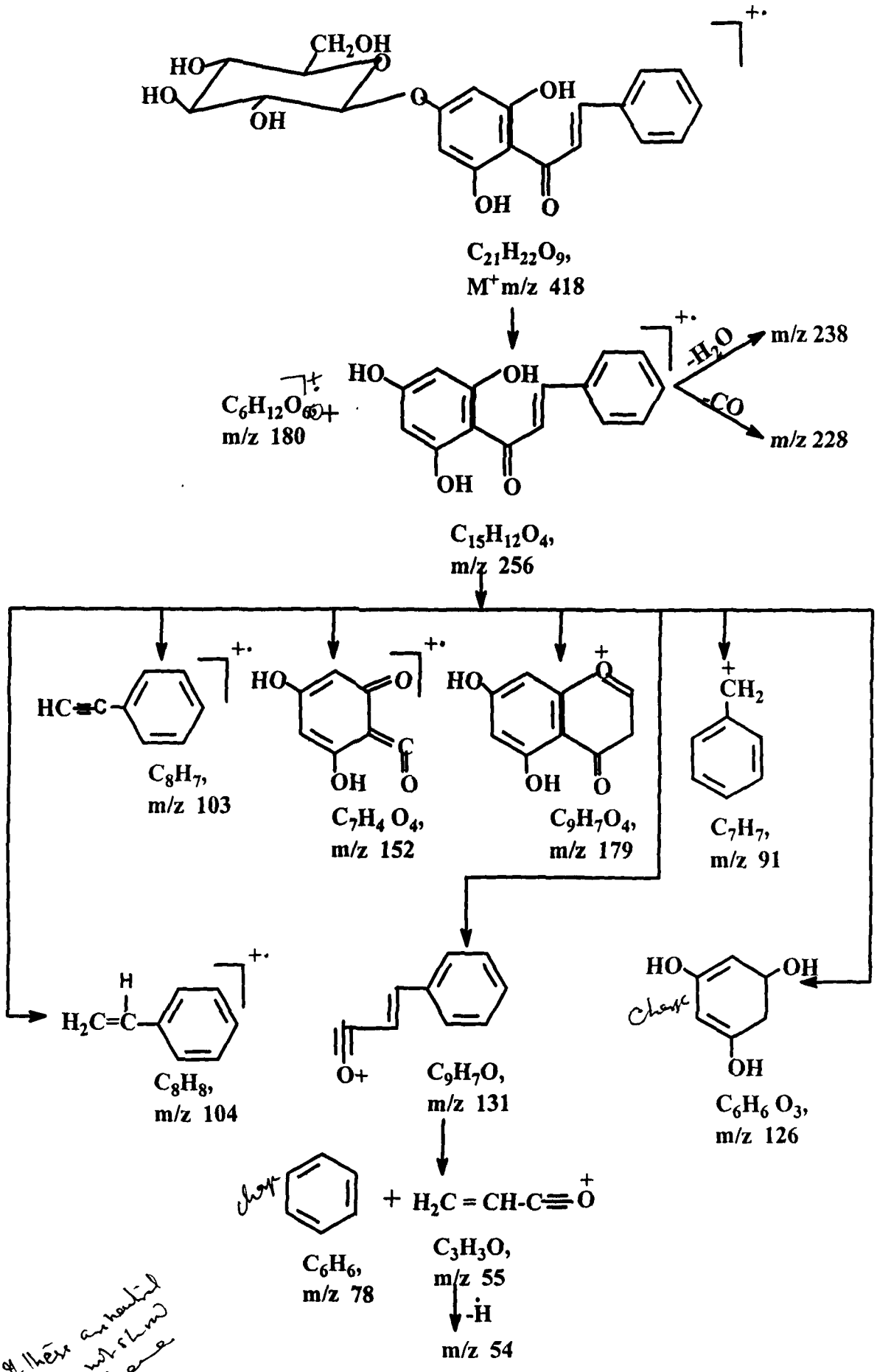


Fig.-XVI


CH2OH
HO
HO
OH
O
OH
OH
O
+·
C21H22O9,
M+ m/z 418
-H2O
m/z 238
-CO
m/z 228
C6H12O6+
m/z 180
C15H12O4,
m/z 256
HC≡C
C8H7,
m/z 103
C7H4 O4,
m/z 152
C9H7O4,
m/z 179
CH2
C7H7,
m/z 91
H2C=C
H
C8H8,
m/z 104
O+
C9H7O,
m/z 131
C6H6 O3,
m/z 126
C6H6,
m/z 78
+ H2C = CH-C≡O+
C3H3O,
m/z 55
-H
m/z 54

(Scheme-IV)

EXPERIMENTAL

The melting points were taken on a Kofler block and are uncorrected. All **ultraviolet** spectra were measured on Beckmann Model DU and Pye Unicam PU-8800 spectrophotometers in methanol / ethanol. **Infrared** spectra were taken on Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The **mass** and **^{1}H-nmr** spectra were obtained from different institutes in the country and outside. The **mass** spectra were mostly measured in E.I. mode on Jeol D-300 while, the **^{1}H-nmr** spectra were usually recorded on Varian EM-360 L (60 MHz), 270 MHz, JEOL 4H-100 MHz, Perkin Elmer R-32 (90 MHz), Brucker dpx 200 MHz, DRX 300 MHz and WM 400 MHz in $CDCl_3$ / DMSO-d_6 using TMS as internal standard.

The silica gel used for different chromatographic purposes, was obtained from E. Merck (India), E. Merck (Germany) and SRL (India). TLC solvent systems used were benzene-pyridine-formic acid (BPF, 36:9:5), toluene-ethylformate-formic acid (TEF, 5:4:1), ethylacetate-ethylmethylketone-acetic acid-water (EtOAc-EtMeCO-AcOH-H_2O, 5:3:1:1; 20:3:1:1; 30:3:1:1), ethylacetate-methanol-water (EtOAc-MeOH-H_2O, 8:1:1), petrol-benzene (2:8) n-butanol-acetic acid-water (BAW, 4:1:5), n-butanol-water-ethanol (BEW, 60:28.5:16.5).

Alcoholic ferric chloride, iodine vapours and aniline hydrogen phthalate solutions were used as spray reagents for visualization of spots on TLC and on paper chromatograms.

STUDY OF THE LEAVES OF ACACIA TORTILIS

The dried and powdered leaves of ***Acacia tortilis*** (3 kg) procured from Yaman, were exhaustively extracted with light petroleum ether (60-80), benzene and finally with methanol. The petrol and benzene concentrate gave positive test for triterpenes.[8] On TLC examination, these concentrates showed number of spots in different solvent systems (Petrol-benzene and petrol-ether) with the same R_f values. The above two concentrates were therefore mixed together. The combined concentrate was chromatographed over silica-gel column, using successively petrol (A), petrol-benzene (9:1-B_1, 8:2-B_2, 7:3-B_3, 6:4-B_4, 1:1-B_5), benzene (C) as eluting solvents. Appropriate fractions (**ir**. Spectra and TLC) were combined.

The fractions A and B_1 on concentration gave a yellowish green oil of fatty nature and was not further examined.

The fractions B_2 and B_3 on TLC examination (silica-gel, petrol-benzene 1:1) showed two major spots with the same R_f values. The above two fractions were therefore mixed together and subjected to column chromatography over silica-gel followed by fractional crystallization, afforded two crystalline TLC homogenous substances, marked as **At-1** and **At-2.**

The fractions B_4, B_5 and C were found to be having the same composition with varying concentrations of the compounds. The three fractions were combined together. Repeated column chromatography over silica gel column using petrol-benzene mixtures in different ratios gave three compounds containing very minor impurities. Several crystallization by appropriate solvent, gave pure compounds labeled as **At-3, At-4** and **At-5.**

The methanol extract was concentrated by heating over a boiling water bath under reduced pressure, a brown gummy mass was obtained. It gave positive colour test for flavonoids. TLC examination of the brown mass in TEF and BPF

systems showed it to be mixture of several compounds. The brown gummy mass was purified by refluxing it with petroleum ether, benzene and chloroform. The semi-solid mass left behind was chromatographed over silica gel column. Fractional elution with benzene-ethylacetate (1:1) and ethylacetate yielded four compounds. They were purified by repeated crystallization and labeled as **At-6, At-7, At-8** and **At-9**. Further elution of the column with ethylacetate-methanol mixture gave two compounds labeled as **At-10** and **At-11.**

At-1:

Elution of the column with petrol-benzene (4:1) afforded a solid substance which on crystallization with benzene-petrol gave white shining crystals **(At-1)** (500mg), m.p 165-66°C, $[\alpha]_D^{20}$ + 24.54 ($CHCl_3$). It gave positive Leiberman-Burchard and Nollers tests and yellow colour with tetranitromethane.

Analysed for $C_{30}H_{48}O$:

Calcd.: C, 84.9; H, 11.37%.

Found: C, 84.7; H, 11.1%

IR, υ^{KBr} cm^{-1}:

3360 and 1030 cm^{-1} (OH), 1630 and 1445 (C=C), 1375 (germinal dim-ethyl), 875 (terminal methylene).

^{1}H-NMR (300 MHz, $CDCl_3$) on δ scale:

0.82 (3H, s), 0.91 (6H, s), 0.93 (3H, s), 0.99 (6H, s), 1.68 (3H, s), 1.34-1.65 (CH_2 – Protons), 4.57 and 4.68 (>C=CH_2), 4.85 (Δ^{12} double bond), 3.20 (1H, m, CH-OH, 3-OH).

^{13}C-NMR (300 MHz, $CDCl_3$) on δ scale:

35.52 (C-1), 25.09 (C-2), 78.88 (C-3), 37.31 (C-4), 55.25 (C-5), 18.26 (C-6), 34.23 (C-7), 39.94 (C-8), 50.38 (C-9), 37.64 (C-10), 20.87 (C-11), 129.63 (C-12), 142.68 (C-13), 47.92 (C-14), 29.76 (C-15), 33.27 (C-16), 48.25 (C-17), 51.15 (C-18), 55.44 (C-19), 150.90 (C-20), 27.37 (C-21), 38.35 (C-22), 31.27 (C-23), 19.25 (C-24), 16.63 (C-25), 16.05 (C-26), 15.34 (C-27), 17.94 (C-28), 109.25 (C-29), 21.04 (C-30).

Mass, m/z:

424 ($M^{+\bullet}$, 100%), 409 ($M^{+\cdot}$-CH_3, 30%), 217 (70.3%), 207 (15%), 205 (20%), 201 (42%), 256 (9%), 190 (29%), 189 (31%), 188 (78%), 174 (35%), 207 (15%).

Acetylation of At-1:

The compound **(At-1)** (60 mg) was treated with pyridine (2 ml) and acetic anhydride (4 ml), allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. The reaction mixture was cooled and poured over crushed ice with constant stirring. Dirty white mass separated was filtered under suction and washed thoroughly with water. The solid thus obtained was crystallized from chloroform-methanol as fine needle shaped crystals (35 mg) m.p 152^0C.

^{1}H-NMR (300 MHz, $CDCl_3$) on δ scale:

0.87 (3H, s), 0.93 (6H, s), 0.96 (3H, s), 1.02 (6H, s), 1.68 (3H, s), 1.36-1.59 (m, CH_2 protons), 4.56 and 4.68 (>C=CH_2), 4.85 (s, Δ^{12}-double bond), 2.17 (3H, s, OAc), 3.17-3.21 (m, CH-OAc, C-3-OAc).

At-2:

At-2 was obtained by the elution of the column with petrol-benzene (7:3) and crystallized with chloroform-methanol as white shining crystals (50 mg), m.p.195°C. Elemental analysis agreed with the molecular formula $C_{30}H_{46}O$.

Analysed for $C_{30}H_{46}O$:

Calcd.: C, 85.30; H, 10.9%.

Found: C, 85.28; H, 10.8%

IR, υ^{KBr} cm^{-1}:

1700 (C=O), 1450 (C=C), 1375 (geminal dimethyl), 845 (terminal methylene, = CH_2)

^{1}H-NMR (300 MHz, $CDCl_3$) on δ scale:

0.79 (3H, s), 0.94 (6H, s), 1.02 (3H, s), 1.03 (3H,s), 1.05 (3H, s), 1.68 (3H, s), 1.3-1.57 (CH_2 protons), 4.52, 4.57 (>C=CH_2), 4.86 (Δ^{12}-double bond), 1.96-2.26 (m, -CH_2).

^{13}C-NMR (300 MHz, $CDCl_3$) on δ scale:

38.52 (C-1), 25.27 (C-2), 206.1 (C-3), 37.62 (C-4), 54.88 (C-5), 19.68 (C-6), 33.81 (C-7), 39.85 (C-8), 50.50 (C-9), 37.36 (C-10), 20.92 (C-11), 129.86 (C-12), 142.55 (C-13), 47.25 (C-14), 29.18 (C-15), 33.32 (C-16), 43.35 (C-17), 46.80 (C-18), 40.64 (C-19), 123.45 (C-20), 27.48 (C-21), 39.30 (C-22), 31.30 (C-23), 21.68 (C-24), 15.93 (C-25), 16.54 (C-26), 14.46 (C-27), 23.66 (C-28), 121.51 (C-29), 26.21 (C-30).

Mass, m/z:

422 ($M^{+\bullet}$, 44%), 407 ($M^{+\cdot}$-CH_3, 41%), 256 (10), 206 (7), 188 (79%), 174 (35%), 176 (100%)

Bromination of lupan-3-ol, 12, 20 diene:

Lupan-3-ol, 12, 20 diene (50 mg) was dissolved in ether (10 ml) and then bromine solution (5 ml) [prepared by dissolving sodium acetate (anhydrous) (1 gm) in acetic acid (glacial) (100 ml) and bromine (9.5 ml) is add] was added gradually with constant shaking under cold conditions 0-5°. When the addition was complete it was kept under the same conditions with occasional shaking for another 15 minutes and then cold water (≈50 ml) was added. Organic matter was extracted with ether washed with water, $NaHCO_3$ (5%) solution, and sodium thiosulphate solution (5%) and again with water successively. The ethereal layer was dried over sodium sulphate anhydrous and then the solvent was evaporated to give a solid mass (40 mg) which gave positive Beilstein test and a negative nitromethane test.

The solid thus obtained was suspended in acetone (20 ml) cooled in an ice bath and then Jones reagent (5 ml) was added dropwise with constant stirring. When the Jones reagent was added the stirring was continued for a further period of 10 minutes and cold water (50 ml) was added. The organic matter was extracted with ether and washed thoroughly with water to make it neutral. The ethereal layer was dried over anhydrous sodium sulphate and then the solvents were removed by evaporation to give a semisolid mass (25 mg) which gave a positive Beilstein test.

The semisolid thus obtained was dissolved in dry ether (20 ml) and acetic acid (5 ml) then zinc dust (2 gm) was gradually added over a period of 30 minutes with constant shaking. When the addition of zinc was complete it was filtered and the filtrate was washed thoroughly with water to remove acid, the ether layer was dried over anhydrous sodium sulphate and the residue was crystallized from

methanol to give the compound **(At-2)** (10 mg) (along with mother liquour having other compounds), mp,. m.m.p. and TLC identical with the sample **(At-2)** obtained from natural sources.

At-3:

At-3 was obtained on elution of the column with petrol-benzene (1:1). After repeated crystallization with chloroform-methanol, it gave white needle shape crystals, m.p. 262-64°C (150 mg).

Analysed for $C_{30}H_{50}O$:

Calcd.: C, 84.50; H, 11.3%.

Found: C, 84.47; H, 11.1%

^{1}H-NMR (200 MHz, $CDCl_3$) on δ scale:

0.72 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.95 (6H, s, $2CH_3$), 1.05 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.25, 1.34, 1.45, 1.52, 1.58 (22 protons, m, $-CH_2$), 2.26-2.41 [3H, m, (C_2-2H and C_4-1H)].

IR, υ^{KBr} cm^{-1}:

2900 (CH, str), 1705 (>C=O), 1455, 1385, 1355, 1170, 1070.

Mass, m/z:

426 (M^+, 92%), 411 (40), 341 (32), 303 (70), 273 (100).

At-4:

At-4 obtained by elution of the column with petrol-benzene (2:3) and crystallized as white needles from chloroform-methanol, m.p. 198°C, R_f = 0.63 (benzene-chloroform, 8:2). It gave positive Leiberman-Buchard test.

^{1}H-NMR (100 MHz, $CDCl_3$) on δ scale:

0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98 (3H, s), 1.0 (3H, s), 1.14 (3H, s), 1.08, 2.01 (-CH_2 and -CH protons of cyclic system and side chain), 3.01 (1H, dd, J=9 Hz and 7 Hz), 4.88 (a broad singlet, 1H, OH proton), 5.21 (1H, m, olefinic proton).

IR, υ^{KBr} cm^{-1}:

3360 (OH), 2960, 2880, 1650, 1465 (C=C), 1040 and 980 cm^{-1}.

Mass, m/z:

426 $[M]^{+\bullet}$

Acetylation of At-4:

The compound **(At-4)** (25mg) was acetylated by heating it with acetic anhydride (1 ml) and pyridine (0.5 ml) on a boiling water bath for 4 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was washed well with water and dried. On crystallization from chloroform-methanol, it gave colourless needles m.p. 241-42°C, $[\alpha]_D^{23}$ + 68.9°.

IR, υ^{KBr} cm^{-1}:

1722 and 1240 (OAc), 1635, 812.

^{1}H-NMR ($CDCl_3$) on δ scale:

0.84 (3H, s, H-28), 0.89 (12H, s, H-23, 24, 29, 30), 0.96 (6H, s, H-25, 26), 1.14 (3H, s, H-27), 2.08 (3H, s, OAc), 4.54 (1H, dd, J=6 Hz, H-3α), 5.20 (1H, t, J=3.5 Hz, H-12).

At-5:

Elution of the column with petrol-benzene (3:7) gave a TLC homogeneous substance which on repeated crystallization from chloroform-ethanol afforded white needle shaped crystals (70 mg) m.p. 136-37°C. It gave an acetate (Ac_2O/py), m.p. 114-15°C, $[\alpha]_D$ – 48.5° ($CHCl_3$) and monobenzoate, m.p. 145-46°C.

IR, υ^{KBr} cm^{-1}:

3340 (OH), 1055, 1655, 840 (C=C), 1460, 1375 (C-Me_2).

^{1}H-NMR (90 MHz $CDCl_3$) on δ scale:

0.70 (3H, s, 18-Me), 0.80 (3H, d, J=6.8 Hz, 28-Me), 0.88 (6H, d, J=6.5 Hz, 26, 27-Me), 0.92 (3H, d, J=6.5 Hz, 21-Me), 1.02 (3H, s, 19-Me), 3.56 (1H, m, 3-ax-H), 5.36 (1H, m, olefinic proton), 1.07-2.34 (-CH_2 and –CH protons of cyclic system side chain).

Mass, m/z:

414 $[M]^{+\bullet}$

Acetylation of At-5:

Crystalline **(At-5)** (30 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a steam bath for 2 hours. After usual work up the solid was washed well with water and dried. On several crystallization from chloroform-methanol it gave colourless flakes (15 mg), m.p. 114-15°C $[\alpha]^{17}_D$ – 48.5°.

Analysed for $C_{31}H_{52}O_2$:

Calcd.: C, 81.57; H, 11.40%.

Found: C, 81.52; H, 11.37%

IR, υ^{KBr} cm^{-1}:

2930, 2850, 1730, 1660, 1460, 1380, 1260, 960.

Benzoate Formation:

The **At-5** (40 mg) was treated with benzoyl chloride (1 ml) and pyridine (0.5 ml), the mixture was allowed to stand at room temperature overnight and then heated for about 6 hours on a water bath. The reaction mixture was cooled and ice cold water was added. The solid thus separated was filtered, washed with aqueous solution of potassium hydroxide (KOH) (2%) and then with water. It was crystallized from methanol, m.p. 145-46°C (25 mg), $[\alpha]_D^{17}$-7.52°.

At-6:

The benzene-ethylacetate fractions (1:1) of the column were found to be identical on TLC and therefore pooled together. Recovery of the solvent gave a gummy mass. On TLC examination it showed the presence of one major compound along with some minor impurities which were removed by crystallization with benzene-acetone, and yellow shining crystals of **At-6** were obtained m.p. 352°C.

Analysed for $C_{15}H_{10}O_5$:

Calcd.:	C, 66.66; H, 3.70%.
Found:	C, 66.78; H, 3.74%

UV with shift reagents, λ_{max} nm:

MeOH	265,297 sh, 338
$AlCl_3$	279, 300, 340, 390
$AlCl_3$/HCl	279, 299, 340, 389
NaOAc	279, 304, 376
NaOAc/H_3BO_3	266, 300 sh, 338.

Acetylation of At-6:

Crystalline **(At-6)** (25 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. After usual work up as described earlier the solid obtained on several crystallization from chloroform-methanol gave colour-less crystals m.p. 183-84°C.

^{1}H-NMR (100 MHz, $CDCl_3$) on δ scale:

7.85 (2H, d, J=9 Hz, H-2',6'), 7.04 (2H, d, J=9 Hz, H-3',5'), 6.60 (1H, s, H-3), 6.66 (1H, d, J=2.5 Hz, H-8), 6.51 (1H, d, J=2.5 Hz, H-6), 2.42 (3H, s, OAc-5), 2.35 (6H, s, OAc-4',7).

At-7:

At-7 was obtained from the fractions obtained by elution of the column with benzene-ethylacetate (1:1). On repeated crystallization with ethylacetate-acetone afforded yellow fine crystals m.p. >315^{0}C were obtained.

Analysed for $C_{15}H_{10}O_6$:

Calcd.:	C, 62.93; H, 3.49%.
Found:	C, 62.94; H, 3.50%

IR, υ^{kBr}_{max} cm^{-1}:

3400 (OH), 1640 (>C=O), 800, 840

UV with shift reagents, λ_{max} nm:

MeOH	258, 265, 292 sh, 346
NaOMe	296, 328 sh, 396
$AlCl_3$	276, 304 sh, 327, 426
$AlCl_3$/HCl	267 sh, 295 sh, 355, 384
NaOAc	291, 326 sh, 377
NaOAc/H_3BO_3	277, 291 sh, 360, 432 sh

Acetylation of At-7:

Crystalline **(At-7)** was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. After usual work up, it was crystallized with chloroform-methanol as colourless needles m.p. 200-201°C.

^{1}H-NMR (270 MHz, $CDCl_3$) on δ-scale:

6.45 (1H, d, J=2.5 Hz, H-6), 6.59 (1H, s, H-3), 6.95 (1H, d, J=2.5 Hz, H-8), 7.25 (1H, d, J=9 Hz, H-5'), 7.75 (1H, dd, J_1=9 Hz & J_2=2.20 Hz, H-6'), 7.80 (1H, d, J=2.20 Hz, H-2'), 2.43 (3H, s, OAc-5), 2.35 (3H, s, OAc-7), 2.33 (6H, s, OAc-3',4').

Mass, m/z:

286 $[M]^{+\bullet}$, 153 $[A_1+H]^{+\bullet}$, 134 $[B_1]^{+\bullet}$

At-8:

It was obtained by elution of column with benzene-ethylecetate (1:1) and was crystallized from methanol as yellow crystals m.p. 311-12^{0}C.

Analysed for $C_{15}H_{10}O_7$:

Calcd.: C, 59.62; H, 3.31%.

Found: C, 59.70; H, 3.33%

UV with shift reagents, λ_{max} nm:

MeOH	256, 270 sh, 301 sh, 372
NaOMe	247 sh, 321 (Dec)
$AlCl_3$	274, 304 sh, 334, 458
$AlCl_3$/HCl	264, 358, 427
NaOAc	257 sh, 274, 329, 390
NaOAc/H_3BO_3	264, 303 sh, 389

Acetylation of At-8:

Crystalline **(At-8)** (20 mg) was acetylated by heating it with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. The reaction mixture was cooled at room temperature and poured on crushed ice. The solid was collected, washed with water and dried. On crystallization from methanol it gave cream coloured m.p. 194-95°C (10 mg).

^{1}H-NMR (100 MHz, $CDCl_3$) on δ-scale:

7.74 (1H, d, J=2.5 Hz, H-2'), 7.63 (q, J_1=2.5 Hz, J_2=8.5 Hz, H-6'), 6.92 (1H, d, J=8.5 Hz, H-5'), 6.87 (1H, d, J=2.5 Hz, H-8), 6.65 (1H, d, J=2.5 Hz, H-6), 2.35-2.40 (15H, m, 5 x OAc).

At-9:

Elution of the column with ethylacetate gave a fraction which on crystallization with methanol afforded pale yellow crystals m.p168^{0}C yield (30 mg). It was analysed for $C_{23}H_{18}O_4$. m.p.

IR, υ^{KBr} cm^{-1}:

2980 (br, OH), 1670, 1480 (C=C)

UV with shift reagents, λ_{max} nm:

MeOH	262, 295, 339
NaOMe	267, 338 (Dec)
$AlCl_3$	272, 299, 367
$AlCl_3$/HCl	273, 369
NaOAc	273, 324
NaOAc/H_3BO_3	269, 296 (Dec)

^{1}H-NMR (200 MHz, DMSO-d_6) on δ-scale:

2.50 (3H, s, CH_3), 2.59 (2H, s, CH_2), 6.17 (1H, d, J=2.5 Hz, H-6), 6.40 (1H, d, J=2.5 Hz, H-8), 6.89 (4H, d, J=9 Hz and 2.5 Hz, H-3',5',3",5"), 7.56 (4H, d, J=9 Hz and 2.5 Hz, H-2',6',2"',6"), 7.86 (1H, s, H-2), 2.59 (2H, s, CH_2), 9.27 (1H, brs, 7-OH), 12.46 (1H, s, 5-OH).

Mass, m/z:

358 ($M^{+\bullet}$) (3.2%), 314 ($M^{+\cdot}$-Co-CH_3-H, 15%), 326 ($M^{+\cdot}$-H_2O-CH_3+H, 10%). RDA fragment 152 (2%), 124 (152-CO, 10%), 91 (100%), 206 (18%).

At-10:

At-10 was crystallized from methanol-chloroform mixture as yellow needles m.p. 263-64°C (250 mg). It gave dark reddish-colour with Mg-HCl, and positive Molish test (2 ml of aq. extract of the compound was added two drops of a freshly prepared 20% alcoholic solution of α-naphthol. The mixture on treatment with 2 ml of conc. H_2SO_4 produced a red-violet ring which disappeared on the addition of an excess of alkali solution) and a dark brown colour with alcoholic $FeCl_3$ solution.

Analysed for $C_{21}H_{20}O_{10}$:

Calcd: C, 56.12; H, 4.67%

Found: C, 56.09; H, 4.62%

IR, υ^{KBr} cm^{-1}:

3420(OH), 2950,1650(C=O)

UV with shift reagents, λ_{max} nm:

MeOH	268, 298, 335
NaOMe	278, 325, 393
$AlCl_3$	279, 301, 353, 384
$AlCl_3$/HCl	276, 300, 351, 385
NaOAc	278, 300, 377
NaOAc/H3BO_3	270, 308 sh, 342

Acetylation of At-10:

The crystalline glycoside **(At-10)** (30 mg), was dissolved in pyridine (1 ml) and acetic anhydride (2 ml) was added. The mixture was heated on a water bath for about 3 hrs. and then left overnight at room temperature. After usual work up, the solid obtained was crystallized from ethylacetate-petroleum ether as white crystals (At-10Ac), m.p. 154-56°C.

Analysed for $C_{33}H_{32}O_{16}$:

Calcd: C, 57.89; H, 4.67%

found: C, 57.79; H, 4.63%

^{1}H-NMR (300 MHz, $CDCl_3$) on δ scale:

1.72-2.02 (12 H, m, 4 x OAc, aliphatic acetoxyls), 2.32, 2.43, 2.51 (9H, s, 3 x OAc, aromatic acetoxyls), 3.65-5.70 (7H, m, H-1",2",3",4",5",6"), 4.64 (1H, d, J=10 Hz, H-1"), 6.8 (1H, s, H-3), 6.91 (1H, s, H-6), 7.4 (2H, d, J=9 Hz, H-3', 5'), 8.1 (2H, d, J=9 Hz, H-2',6').

Hydroiodic acid oxidation:

A mixture of the glycoside **(At-10)** (30 mg), phenol (70 mg) and hydroiodic acid (0.3 ml) was refluxed for about 9 hours. The mixture was cooled and sodium bisulphite ($NaHSO_3$) was added to it with stirring. The separated brown substance was purified by passing it through a silica gel column.

Elution of the column with benzene-ethylacetate (1:1) mixture afforded the substance, At-10ag. It was crystallized from chloroform-ethylacetate as light yellow crystals.

Yield (15 mg), m.p. 347-48°C.

Analysed for $C_{15}H_{10}O_5$:

Calcd: C, 66.66; H, 3.70%

Found: C, 66.64; H, 3.54%

UV data with shift reagents, λ_{max} nm:

MeOH	266, 297, 336
NaOMe	277, 326, 395
$AlCl_3$	280, 303, 351, 382
$AlCl_3$/HCl	278, 300, 352, 384
NaOAc	276, 300, 378
NaOAc/H_3BO_3	270, 306 sh, 345

Ferric chloride oxidation:

The glycoside **(At-10)** (30 mg) was added to a solution of $FeCl_3$ (120 mg) in 3 ml water. The mixture was heated on an oil bath at 125°C for about 7 hours. The reaction mixture after cooling, was diluted with water (10 ml), a small amount of dark coloured solid formed was filtered off. The filtrate was purified by passing through a column of silica gel using water as eluant. The initial fractions obtained were combined and concentrated to a syrup which was subjected to paper chromatography on Whatman No. 1 filter sheet using n-BuOH-AcOH-H_2O (4:1:5) and n-BuOH-water-ethanol (60:25:8:16.5) as solvent systems and employing the descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hrs. and after drying at room temperature were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-105°C revealed the presence of glucose only.

GLC of Trimethyl silyl ether of sugar:

The TMSi ether of sugar was prepared by taking 15 mg of sugar in dry pyridine (0.5 ml) and hexamethyl disilazane (0.2 ml) in a 10 ml round bottom flask. To this solution 0.2 ml of trimethyl chlorosilane was added. The flask was stoppered and kept at room temperature for one hour. The solution after drying was taken in heptane. The heptane soluble TMSi ether derivative of sugar was then subjected to glc (2% OV-1, column temp. 150-250°C, 10 min. dect. temp. 300°, N_2, 50 ml/min) along with the silyl derivative of standard sugar. The observed Rt. value was found to be in agreement with that of authentic sample of glucose (Rt. glucose 1.0).

Periodate oxidation of glycoside methyl ether:

Glycoside methyl ether (15 mg) of **At-10** was dissolved in methanol (10 ml) and an aqueous solution of $NaIO_4$ (0.47 N, 15 ml) was added to it. The mixture was kept at 20°C in dark for 24 hours. Solid $NaHCO_3$ (2 gm) was then added followed by the addition of Na_3AsO_3 solution (0.05 N, 25 ml). The resultant mixture was titrated against iodine using starch as indicator. One mole of methyl ether consumed 1.2 mole of periodate, with the liberation of one mole of formic acid.

At-11:

Fractions obtained by the elution of the column with ethylacetate-methanol (8:2-7:3) gave pale yellow solid. It was crystallized from methanol-chloroform as pale yellow crystals (160 mg), m.p. >280.

Analysed for $C_{21}H_{22}O_9$:

Calcd: C, 60.28; H, 5.26%

Found: C, 60.23; H, 5.20%

IR, υ^{KBr} cm^{-1}:

2965 (chelated OH), 1684 (C=O), 1462 (C=C)

UV data with shift reagents, λ_{max} nm:

MeOH	245, 278, 322 sh, 365
NaOMe	244, 271, 324 sh, 366
$AlCl_3$	246, 284, 320 sh, 435
$AlCl_3$/HCl	246, 282, 325 sh, 436
NaOAc	245, 277, 324 sh, 366
NaOAc/H_3BO_3	245, 276, 323 sh, 365

^{1}H-NMR (300 MHz, DMSO) on δ scale:

6.24 (1H, d, J=2.0 Hz, H-3'), 6.26 (1H, d, J=2.0 Hz, H-5'), 6.77-6.85 (5H, m, H-2,3,4,5,6), 6.97 (1H, d, J=15 Hz, H-α), 7.85 (1H, d, J=15 Hz, H-β), sugar protons: 4.1 (1H, d, J=9 Hz, H-1"glu), 3.5-4.32 (4H, m, H-1",2",3",4"), 5.22-5.50 (3H, m, H-5",6"), 13.60 (1H, s, 2'-OH), 12.85 (1H, s, 6'-OH).

Mass, m/z:

$M^{+\cdot}$ 418 (18%), 256 (10.4%), 238 (6.4%), 228 (6.8%), 104 (8.5%), 103 (15%), 152 (10.8%), 179 (6.3%), 131 (7.4%), 91 (10%), 126 (16.4%), 55 (7.8%), 54 (7.5%), 78 (6%), 180 (10.6%).

Acetylation of At-11:

At-11 (30 mg), was dissolved in pyridine (1 ml) and acetic anhydride (2 ml) was added. The mixture was heated on a water bath for about 3 hrs. After usual work up, as described earlier, the acetate obtained was crystallized from chloroform-methanol as fine needle sharped crystals (15 mg), m.p. 178^{0}C.

^{1}H-NMR (200 MHz, $CDCl_3$) on δ scale:

1.78-2.05 (12H, m, 4 aliphatic acetoxyls), 2.52 (6H, s, aromatic acetoxyls OAc-2',6'), 6.24 (1H, d, J=2.0 Hz, H-3'), 6.26 (1H, d, J=2.0 Hz, H-5'), 6.77-6.85 (5H, m, H-2,3,4,5,6), 6.97 (1H, d, J=15 Hz, H-α), 7.85 (1H, d, J=15 Hz, H-β), 4.1 (1H, d, J=9 Hz, H-1" glu), 3.5-4.32 (4H, m, H-1",2",3",4"), 5.22-5.50 (3H, m, H-5",6").

Acid Hydrolysis of At-11:

The glucoside **(At-11)** (60 mg) was hydrolysed by heating with 6% aqueous HCl on a water bath. The heating was continued for 2 hrs to ensure complete hydrolysis. The mixture was left overnight. The aglycone which settled down was filtered, washed with water and dried. It was crystallized with methanol-chloroform as yellow crystals yield (35 mg) m.p 176^{0}C.

^{1}H-NMR (DMSO) on δ scale:

6.20 (H, d, J=2.0 Hz, H-3'), 6.23 (1H, d, J=2.0 Hz, H-5'), 6.75-6.82 (5H, m, H-2,3,4,5,6), 6.95 (1H, d, J=15 Hz, H-α), 7.81 (1H, d, J=15 Hz, H-β), 13.0 (1H, s, 2'-OH), 12.50 (1H, s, 2'-OH), 10.1 (1H, s, 4'-OH).

UV data with shift reagents, λ_{max} nm:

MeOH	251, 298 sh, 368
NaOMe	253, 280 sh, 319 sh, 346 sh, 430
$AlCl_3$	255, 321, 385 sh, 423
$AlCl_3$/HCl	319 sh, 378 sh, 421
NaOAc	282 sh, 341, 350 sh, 392
NaOAc/H_3BO_3	286, 353 sh, 381, 443

Acetylation of aglycone:

Aglycone (12 mg) was acetylated by heating with pyridine (1 ml) and acetic andydride (2 ml) over water bath for 3 hour. After usual work up it was crystallized with chloroform-methanol as white needles, m.p 155-56^{0}C.

^{1}H-NMR ($CDCl_3$) on δ scale:

2.45 (6H, s, OAc-2',6'), 2.34 (2H, s, OAc-4'), 6.22 (1H, d, J=2.0 Hz, H-3'), 6.25 (1H, d, J=2.0 Hz, H-5'), 6.78-6.83 (5H, m, H-2',3',4',5',6'), 6.99 (1H, d, J=16.0, H-β).

Degradation of aglycone:

The aglycone (10 mg) was dissolved in 50% KOH (2 ml). The mixture was heated over water bath for 3 hrs. it was cooled and acidified by HCl. The solution was extracted with ether. And ether layer was washed with water to remove excess of HCl, then was shaken by $NaHCO_3$ solution, aqueous and organic layers were separated. The ether was dried by passing over anhydrous sodium sulphate and evaporated, the residue on co-TLC examination with an authentic sample of pholoroglucinol showed it to be pholoroglucinol.

The aqueous layer was acidified by adding HCl and extracted with ether. The ether layer was washed with water and dried with sodium sulphate. The identity of the residue was checked by TLC and co-TLC with an authentic sample of cinnamic acid.

Identification of Sugar:

The hydrolysate was concentrated and neutralized over KOH under vacuum and chromatographed on Whatman (No.1) filter paper using n-butanol-acetic acid-water (4:1:5 v/v/v) as the developing system, employing the decending technique. Authentic sugars were used as checks. The chromatogram was run for 24 hours,

after developing, it was dried at room temperature and sprayed with aniline hydrogen pthalate solution heated at 100-5^{0}C for 10 minutes.

The sugar was identified as glucose (R_f 0.18) by comparison with authentic sugar (R_f, co-paper chromatography).

Estimation of Sugar:

The anhydrous glucoside (30 mg) was hydrolysed by refluxing with 2% H_2SO_4 for 2 hours. After cooling over night the aglycone was filtered and dried. The ratio of the aglycone to the glycoside was found to be (1:2) indicating the presence of 1 mole of sugar per mole of the aglycone.

REFERENCE

1. 'The wealth of India', Raw materials, Vol. I., p.4. . .

2. Harsh and H.C. Bohra, **Botany Study Center**, ICAR Publication No.25, pp.1-11 (1985).

3. R.W. Wrangham, P.G. Waterman, J. of **Animal Ecology, 50**, 715-731 (1981).

4. M. Hagos, G. Samuelsson, L. Kennel, B.M. Modawi, **Plant Medica, 53**, 27-31 (1987).

5. H. Thieme and A. Khogali, **Pharmazie, 29**, 352 (1974).

6. Ram P. Rastogi and B.N. Mehrotra, **'Compendium of Indian Medicinal Plants**, Vol.5, p.6 (1998).

7. L. Prakash and Mukhtair Singh, **J. Ind. Chem. Soc.,** Vol. IXIII, Sep. (1986).

8. C.R. Noller, R.A. Souith, G.H. Harris and J.W. Walker, **J. Amer. Chem. Soc., 64**, 3027 (1962).

9. M.S. Alam, Neeraj Chopra, M. Ali and M. Niwa, **Phytochemistry, 54**, 215-220 (2000).

10. W.Z. Zimmermann, **Phytochemistry,** 2337, 257 (1935).

11. A.A. L. Geirasatilika, Y.M.A. De-J, Silva, S. Sotheeswaran, S. Balasabramonian, M.M. Wazeer, **Phytochemistry, 23**, 323-8 (1984).

12. M. Crawford, S.W. Hansan, M.E.S. Kokar, **Tetrahedron Letters**, 3099 (1975).

13. H. Budzikiswiez, J.M. Wilson, C. Djerassi, **J. Amer. Chem. Soc., 85**, 3688 (1963).

14. A.R. Irvine, **'Wooding Plants of Ghana'**, p.362 (1961).

15. T. Inoue, Y. Ishidata, M. Fujita, M. Kubo, M. Fukushina and M. Nagai, **Yakugakee Zasshi, 98(4)**, 41-46 (1978).

16. J.B. Harbone, '**Comparative Biochemistry of the Flavonoids'**, Academic Press, London (1967).

17. J. Shinoda, **J. Chem. Pharm. Soc.,** Japan **48**, 214 (1928).

18. T.J. Mabry, K.R. Markham and M. B. Thomas, **'The Systematic Identification of Flavonoids'**, Springer, New York (1970).

19. V.M. Chari, R.J. Grayer-Barkmerjer, J.B. Harbone and G. Osterdahi, **Phytochemistry, 20**, 1977 (1981).

20. T.A. Geissman, **'The Chemistry of Flavonoid Compounds'** (edit Pergamon Press, Oxford, London), p.72 (1964).

21. Syed M. Ahmed, **Ph.D. thesis**, Aligarh Muslim University, p.81 (1988).

22. R.M. Horowitz and B. Gentili, **Chem. Ind.,** 498 (1964).

23. Batterham T.J. and R.H. Highet, **Aust. J. Chem., 17**, 428 (1964).

24. A. Prox, **Tetrahedron Letters,** 3697 (1968).

25. K. Venkataram, **'The Chemistry of Flavonoid compounds',** (ed. T.A. Geissmann, Perganon Press, London), p.72 (1962).

26. K.R. Markham and V.M. Chari in **'The Flavnoids Advances in Research',** (eds. J.B. Harbone and T.J. Mabry) p.32 (1982).

27. T.J. Mabry, K.R. Markham, and M.B. Thomas, **'The systematic identification of the Flavonoids',** pp.228-229.

28. Hillis, W.S. and G. urbach, **Nature, 182,** 657 (1982).

29. F. Bohlmann and W.R. Abraham, **Phytochemistry, 18**, 1754 (1979).

30. M. Somogyi, **J. Biol. Chem., 19**, 195 (1952).

31. L.H. Briggs and R.H. Loeker, **J. Chem. Soc.,** 1859 (1949).

CHAPTER-III

LANNEA ACIDA

Results &

DISCUSSION

CHEMICAL CONSTITUENTS FROM THE BARK OF LANNEA ACIDA RICH (ANACARDIACEAE)

Lannea acida (syn. **Odina acida)** a small deciduous tree, leaves exceeding 30 cm. It is used as an important drug of the indigenous system of treatment in North Nigeria. The leaves and bark are used as febrifuge and have been described to be useful in gout, rheumatism, for wounds, swelling and burns[1]. A survey of the literature showed that no work has been reported on the bark of this medicinally important plant[2], therefore the present discussion deals with the isolation and characterization of the following compounds from the bark of **Lannea acida**.

1. **6,7-(2",2"-dimethyl chromeno)-8-γ,γ-dimethyl allyl flavanone**

2. **3',4' dihydroxy-7,8 (2",2"-dimethyl chromeno)-6-γ,γ dimethyl allyl flavonol.**

3. **7-methyltectorigenin**

4. **Irisolidone**

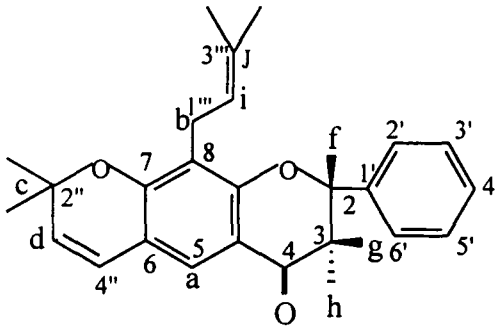
Coarsely powdered bark (1 Kg) was exhaustively extracted with acetone. The acetone concentrate was successively extracted with petroleum ether, chloroform and finally with ethylacetate. The chloroform and ethylacetate concentrates gave positive tests for flavonoids.[3-6] On TLC examination these concentrates showed four major spots in BPF and TEF, with the same R_f values. The above two concentrates were therefore mixed together. Repeated column chromatography over silica gel followed by fractional crystallization afforded four crystalline TLC homogeneous substances. They were given the labels as **La-1, La-2, La-3 and La-4** in order of their increasing R_f values.

La-1:

La-1 was eluted from the column by benzene-ethylacetate (3:1) mixture. On crystallization from benzene-ethylacetate it gave colourless needles m.p. 92^0C. Elemental analysis and the molecular ion peak at m/z 374 agreed with the molecular formula $C_{25}H_{26}O_3$. A pink colour with magnesium and hydrochloric acid and a blue colour on treatment with sodium amalgam followed by acidification, suggested a flavanone nucleus for the compound.[7] In its **^{1}H-nmr** spectrum **(Fig.I)** two protons (C_3–2H) multiplet centered around δ 3.0 and a one proton (C_2–H) multiplet centered around δ 5.15, clearly defines **La-1** as a flavanone.

The **^{1}H-nmr** spectrum of **La-1** showed the presence of a γ,γ-dimethyl allyl unit and 2",2"dimethyl chromene ring. The singlet at δ 1.46 (c) for six protons and two olefinic doublets at δ 4.4(e) and δ 3.4(d) with Jde 11 Hz signify the dimethyl chromene ring. The two three protons singlets at δ 1.75 and 1.8 (J) and the doublet at δ 3.2 (b) (J=7 Hz) for two protons and an olefinic triplet, at δ 5.4 (i) (J=7 Hz) revealed the γ,γ-dimethyl allyl unit. Besides these a sharp singlet at δ 7.5 for five protons (unsubstituted phenyl) and the singlet at δ 7.7(a) for one proton (C_5–H) indicated that the compound has a trisubstituted. Ring-A having the chromene as well as the dimethyl allyl unit and the unsubstituted side phenyl.

On the basis of the findings of the **^{1}H-nmr** spectrum. **La-1** was assigned the structure as **6,7-(2",2"dimethyl chromeno)-8-γ,γ-dimethyl allyl flavanone (I).**



(I)

In the **^{1}H-nmr** spectrum **(Fig. I),** the chromanone ring hydrogens f.g and h showed the signals centered at δ 5.35, δ 2.8 and δ 3.0 respectively, coupling constant J gh 18, J fh 15 and J f g 4 Hz.

The **mass** spectrum **(Fig.II)** of **La-1** showed a molecular ion peak at m/z 374 (10%). The base peak appears at m/z 55. The **(scheme-I)** outlines the pattern of fragmentation. The, peak at m/z 359 belong to the ion arising by the loss of one of the methyl groups at chromene ring. A peak at m/z 319 showed the loss of C_4H_{17}. This is in analogy with the cleavage of the side chain as scandenone-4-methyl ether.[8]

Fig.-I

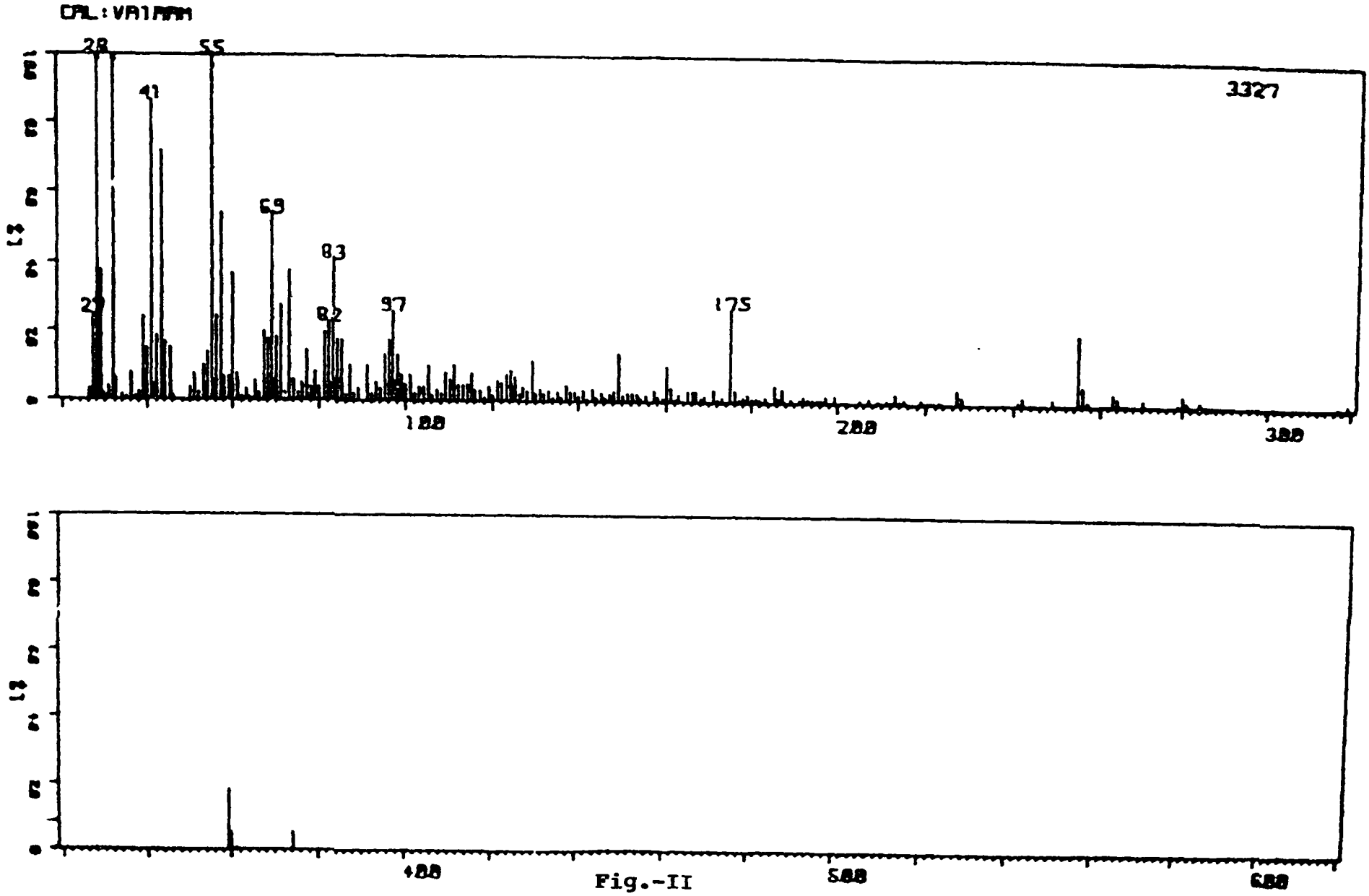
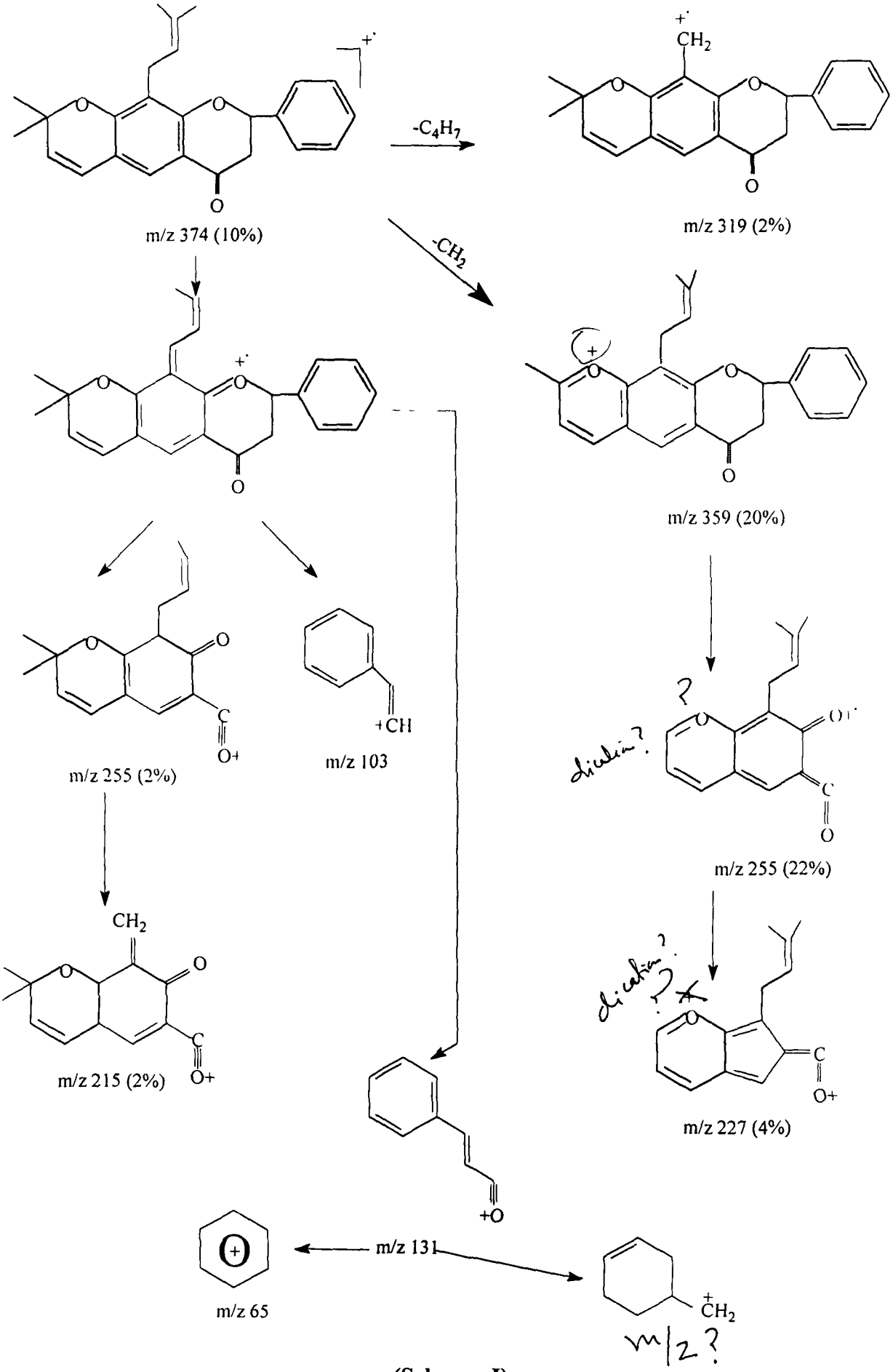


Fig.-II


-C4H7
m/z 374 (10%)
m/z 319 (2%)
-CH2
m/z 359 (20%)
m/z 255 (2%)
m/z 103
m/z 255 (22%)
m/z 215 (2%)
m/z 227 (4%)
m/z 131
m/z 65

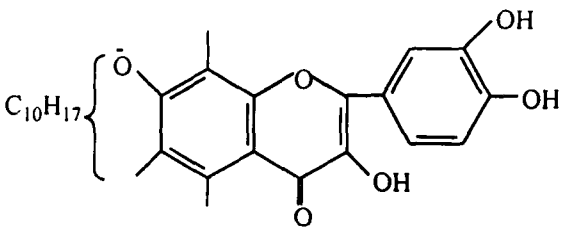
(Scheme -I)

La-2:

The fraction **La-2** was eluted with benzene-ethylacetate mixture (1:1). Recovery of the solvent left a residue, which was crystallized from methanol as yellow needles, m.p.165^{0}C. Elemental analysis suggested $C_{25}H_{24}O_6$ as the molecular formula of the compound, further confirmed by the molecular ion peak at m/z 420.

The **uv** and **ir** spectra of **La-2** and its derivatives showed that it contained a conjugated carbonyl group and three phenolic hydroxyl groups. It gave green colour with ferric chloride. A pink colour with magnesium and hydrochloric acid[9] and a bright yellow colour with Wilson-boric acid reagent[10] showed it to be a flavonol. A flavonol structure was evidenced further with the **uv** data for **La-2** and its derivatives and with the marked dependence of the **ultra-violet** spectrum of **La-2** upon solvent and pH.[11] A 20 nm red shift of Band I in the presence of NaOAc / H_3BO_3 indicated the presence of ortho-dihydroxy group. Alkaline fusion gave 3,4-dihydroxybenzoic acid.

Assuming a flavonol structure for **La-2**, five oxygen atoms were thus accounted for, two belonging to the γ-pyrone ring and three to phenolic hydroxyl groups. This led to the proposal of the partial structure (II-a). On biogenetic grounds and by inspection of the **uv** data, the sixth oxygen was placed in position 7.

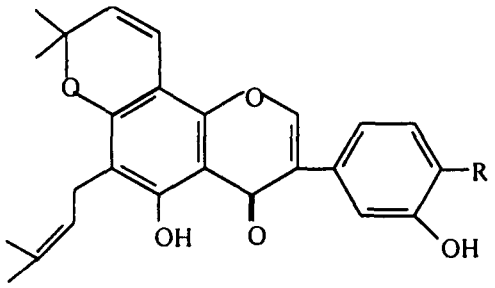


(II-a)

Biosynthetic argument suggest that the $C_{10}H_{17}$ residue in **(II-a)** may consist of two isoprene units[12] (C_5H_8+ C_5H_9).

La-2 and its derivatives showed absorption in the 222-230 nm region which is characteristic of 2",2"dimethyl chromene. Kuhn-Roth oxidation of **La-2** gave 1.25 equivalent of acetic acid, which suggested two Me_2C units. Thus the C_5H_9 unit could be located in a 2",2"-dimethyl chromene residue and a C_5H_9 unit could exist as a CMe_2: $CH\text{-}CH_2$ group.

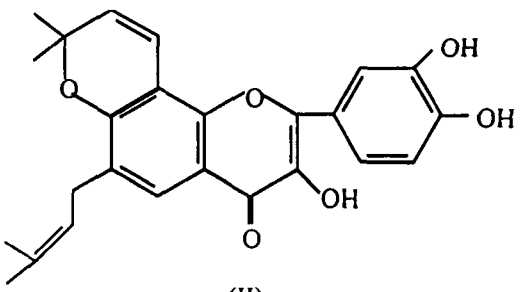
Following structure (II) was tentatively proposed [cf[8] osajin (II-a) & pomiferin (II-b)].



II-b: R = H
II-c: R = OH

The **^{1}H-nmr** spectrum **(Fig.III)** of the acetate of **La-2** exhibited a singlet at δ 1.4 for six protons, two doublets at δ 5.8 and 6.7 (J=10 Hz) signifying the dimethyl chromene ring. Two singlets for three protons each at δ 1.8 and δ 1.9, doublet at δ 3.4 (J=7 Hz) for two protons and an olefinic proton multiplet at δ 5.2 revealed the γ,γ-dimethylallyl unit.[13,14,15] In addition the spectrum showed one proton singlet at δ 7.9 ascribable to C_5–H.

On the basis of above data, **La-2** was assigned the structure as **3',4' dihydroxy-7, 8-(2",2"-dimethyl chromeno)-6-γ,γ-dimethy allyl flavonol (II)**.



(II)

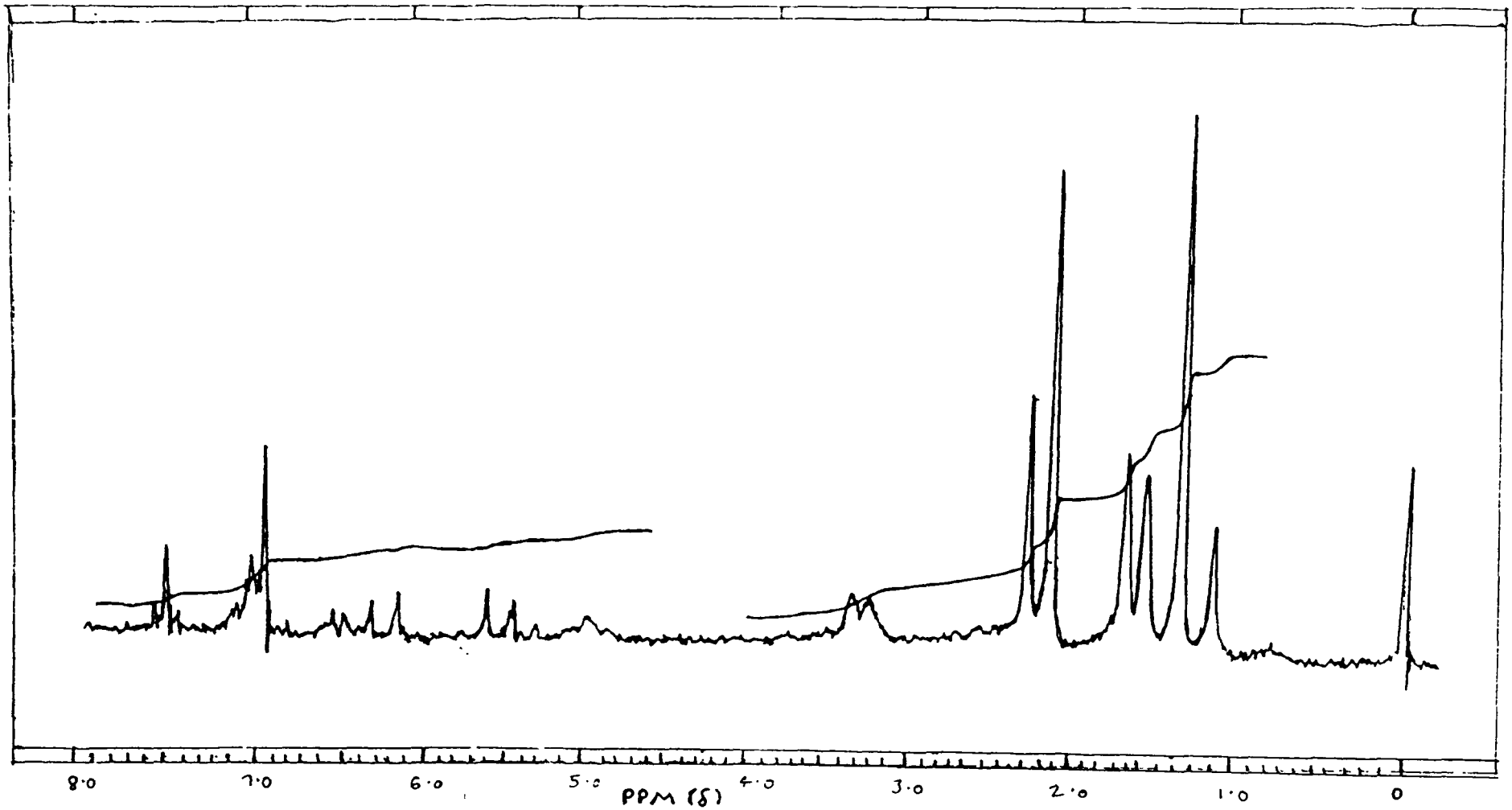


Fig.-III

La-3:

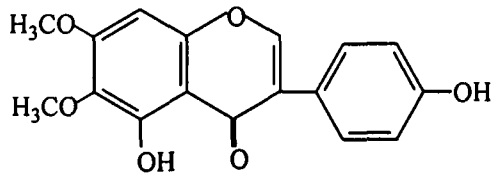
Fraction **La-3** was obtained from benzene-ethylacetate mixture (1:3) and crystallized as yellow needles from methanol. The molecular ion peak at m/z 314 and elemental analysis point the molecular formula as $C_{17}H_{14}O_6$ Micro-Zeisel determination showed the presence of two methoxyl groups. The presence of two free hydroxyl groups was confirmed by the formation of diacetate and dimethyl ether of the compound **La-3**.

It gave pink colouration on treatment with sodium amalgam followed by acidification with HCl. The **uv** spectrum showed λ_{max} at 268 nm and an inflection at 334 nm. The molecular formula, positive colour test with sodium amalgam and **uv** absorption suggest it to be an isoflavone, further confirmed by the **^{1}H-nmr** in which the signlet of the C-2 proton appeared at about δ 7.8. A dark green colour with ferric chloride[16] and a band at 3450 cm^{-1} in **ir** spectrum showed the presence of chelated hydroxyl group, further confirmed by a red shift of 12 nm in the **uv** spectrum on addition of anhydrous aluminum chloride.

The formation of di-acetate and dimethyl ether, along with the molecular formula and **uv** spectra suggest that the compound is an isoflavone with two methoxyl and two hydroxyl groups. The methanolic solution of the compound was not oxidised by pentamine cobalttrichloride, indicating the absence of adjacent phenolic hydroxlic groups. One of the hydroxyl group, was placed at C-5. In the **^{1}H-nmr** spectrum the aromatic region contains multiplets of four protons of ring B. A multiplet and a doublet centered at δ 6.9 and δ 7.4 respectively corresponds to an A_2B_2 pattern, the remaining hydroxyl group was placed at C-4', which funds by mass fragmentation.

In the **^{1}H-nmr** spectrum **(Fig.-IV)** a sharp singlet at δ 7.8 indicated the presence of C-2 proton of γ-pyrone nucleus. The presence of two methoxyl groups, was indicated through two singlets at δ 3.92 and 3.96 for three protons each. A singlet at δ 6.46 integrating for one proton can be assigned to an aromatic proton shielded by two ortho and one paraoxygen. It can arise from

the C-6 proton of a 5,7,8-trioxygenated isoflavone or the C-8 proton of 5,6,7-tri oxygenated isoflavone. The methoxyl has been put at 6-position on the evidence of **mass** spectrum, thus assigning the singlet at δ 6.46 to C-8 proton.



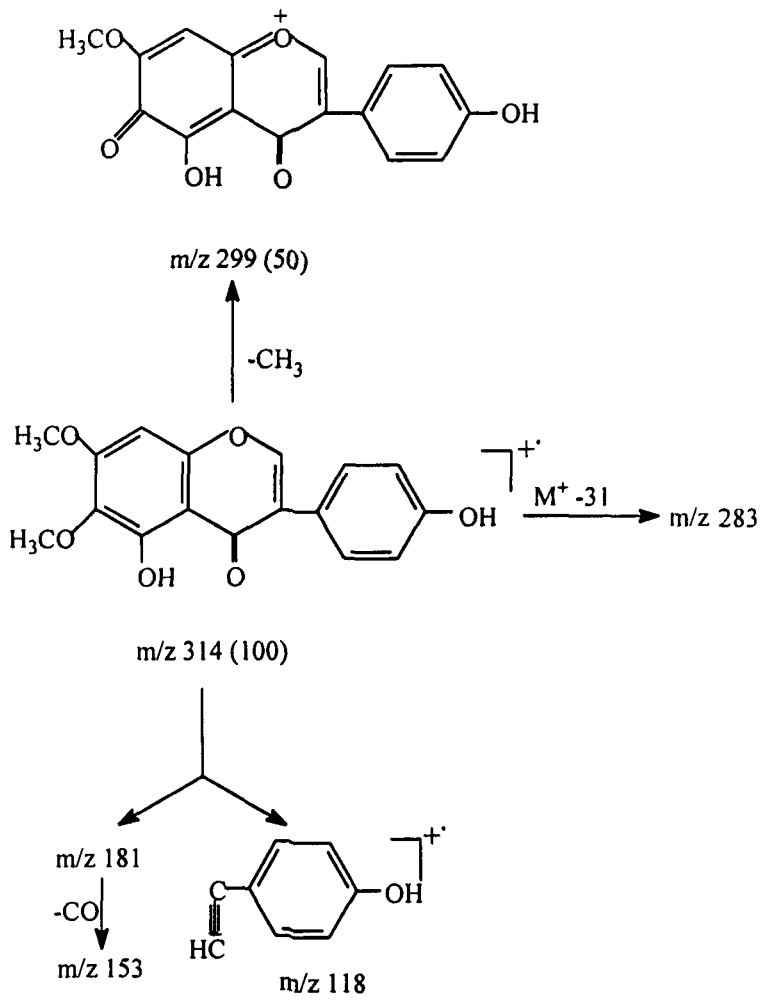
(III)

The compound **La-3** was characterized as **7-methyltectorigenin (III)** by its melting and mixed melting points with an authentic sample of 7-methyl-tectorigenin and its acetate.[17] Further conformation to its identify was furnished by spectral evidences.

The **mass** spectrum **(Fig-V)** showed $M^{+\cdot}$ at m/z 314 and $M^{+\cdot}$–15 corresponding to the loss of methyl, at m/z 299.$M^{+\cdot}$ is 100% and $M^{+\cdot}$–15 peak is about 70%. This is extremely significant and provides the justification for putting the methoxyl at C-6 for in 8-methoxy 5-hydroxy flavonoids the order is reversed and the predominnt peak is that resulting from the loss of methyl from $M^{+\cdot}$. Peak at m/z 118 suggested it due to p-hydroxyphenylacetylene ion indicating mono oxygenation in ring-B **(scheme-II)**. Ring-A (RDA) fragment expected at 196 is not found but peak at m/z 153 might be coming from 196 by loss of CO.

Fig.-IV

Fig.-V


H3CO
O
OH
O
OH
m/z 299 (50)
-CH3
H3CO
H3CO
OH
O
OH
M+ -31
m/z 283
m/z 314 (100)
m/z 181
-CO
m/z 153
HC
OH
m/z 118

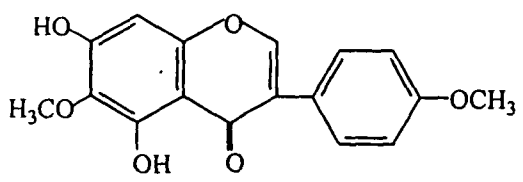
(Scheme-II)

La-4:

La-4 was eluted from the column by benzene-ethylacetate (1:15) mixture. It gave the characteristic colour reactions of isoflavones[10,18].The **uv** absorption spectrum was similar to that of irosolone and tri-O-methyl-tectorigenin.[14] The elemental analysis showed a molecular formula $C_{17}H_{14}O_6$ and two methoxyl groups. A blue colour with ferric chloride and formation of diacetate and dimethyl ether showed the presence of two free hydroxyl groups. Specific colour reaction further indicated that one of these must be located in the 5-position and the vicinal hydroxyl groups were not present. A red shift of 10 nm in the **uv** spectrum characteristic of 5-hydroxyl was observed on addition of aqueous aluminum chloride[19,20] and a similar shift of 8 nm on addition of fused sodium acetate suggested a 7-hydroxyl group.

The **^{1}H-nmr (Fig-VI)** showed a sharp singlet at δ 7.9 for one proton characteristic of the H-2 of the isoflavone. Two singlets at δ 3.8 (3H) and δ 3.9 (3H) indicated the presence of two methoxyl groups. Examination of the aromatic protons region showed the typical A_2B_2 pattern by a doublet centered at δ 6.9 (J=10 Hz) and another centered at δ 7.5 (J=10 Hz), each integrating for two protons, thus showing 4'-oxygenation with side phenyl. A singlet at δ 6.5 (1H) may be assigned to H-8. Thus the compound **La-4** appeared to be a dimethyl ether of 5, 6, 7, 4'-tetrahydroxy isoflavone.

The spectral evidence showed the compound to be **irisolidone**[21] (IV) further confirmed by a comparison of its **uv** and **ir** with an authentic sample of irisolidane and by co-TLC and mixed melting point.



(IV)

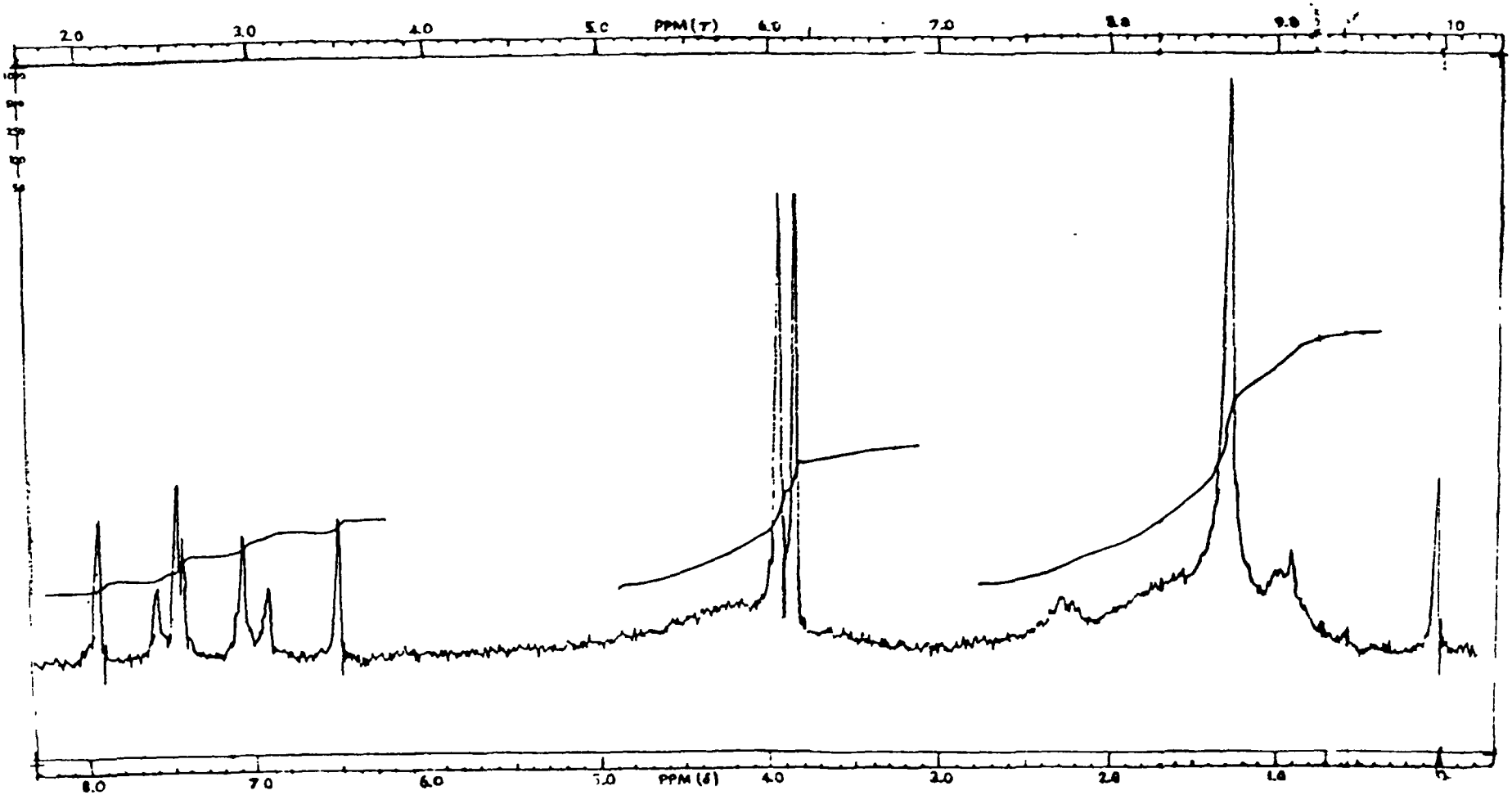


Fig.-VI

EXPERIMENTAL

STUDY OF BARK OF LANNEA ACIDA RICH

Coarsely powdered bark (1 Kg) was exhaustively extracted (3 times, 5 liters each) by refluxing with acetone. All the acetone extracts were combined together and distilled under reduced pressure. A dark brown syrupy mass was left behind. The residue was successively extracted with petroleum ether (60-80^0), chloroform and finally with ethylacetate. The chloroform and ethylacetate concentrates on TLC examination in BPF and TEF systems showed four major spots with the same R_f values and shad in **uv** light. The above two concentrates were therefore mixed together. The combined extract (23 gm) was subjected to column chromatography over silica gel (2.5 Kg) and eluted with benzene-ethylacetate in different proportions, monitored by TLC. Fractions each of 50 ml, were collected and the following compounds were isolated from different pools of identical fractions

La–1 (435 mg)

La–2 (350 mg)

La–3 (250 mg)

La–4 (270 mg)

La-1:

The benzene-ethylacetate (3:1) fractions of the column were found identical on TLC and therefore pooled together. Removal of the solvent by distillation under reduced pressure gave a gummy mass. The gummy mass (7 gm) was dissolved in a small quantity of acetone and mixed with silica gal. The slurry was loaded on a column of silica gel (60 g / 100-200 mesh; 42 x 3.0 cms) and successively eluted with petroleum ether and benzene and finally with benzene-ethylacetate (95:5) and (9:1) mixture. Fractions eluted with benzene-ethylacetate mixture showing single spot on TLC examination were combined together and on concentration gave a dirty white solid which was crystallized from benzene-ethylacetate as white needles melting point 92^0C. λ^{EtoH}_{max} nm (E x 10^{-3}) : 222 (25.5), 272 (14.6)

Analysed for $C_{25}H_{26}O_3$:

Calcd.: C, 80.21; H, 6.95%

Found: C, 80.14; H, 6.97%

^{1}H-NMR (60MHz, $CDCl_3$) on δ scale :

1.5 (6H, s, >C $(CH_3)_2$), 1.75, 1.8 (2x3H, s, >C $(CH_3)_2$), 3.2 (2H, d, J=7 Hz,-CH–), 5.4 (1H, t, =CH–), 5.6, 6.7 (2x1H, d, J=11 Hz -CH = CH-), 7.5 (5H, s, 2',3',4',5'-H), 7.7 (1H, s, 5-H), 3.0 (2H, m, 3–H), 5.15 (1H, m, 2–H).

Mass, m/z:

$M^{+\cdot}$ 374 (10%), 55, 359, 319.

Chalcone:

La-1 (100 mg) was suspended in water (10 ml) and a slow current of nitrogen free from oxygen was passed through the suspension. After 5 minutes aqueous sodium hydroxide (5 ml, 10%) was added, and the mixture was heated over a boiling-water bath for 30 minutes, cooled to room temperature, acidified

with hydrochloric acid, and extracted with ether. The ether extract was dried (Na_2SO_4) and evaporated. The yellow residue was crystallized from ethyl-acetate melting point 125-26^{0}C.

Analysed for $C_{25}H_{24}O_3$:

Calcd.: C, 80.64; H, 6.45%

Found: C, 80.75; H, 6.49%

La-2:

It was crystallized from methanol as yellow needles, m.p. 165^{0}C.

Analysed for $C_{25}H_{24}O_6$:

Calcd: C, 71.42; H, 5.71%

found: C, 7.44; H, 575%

UV., λ_{max}:

MeOH: 225, 295

$NaOAc/H_3BO_3$: 225, 314

IR, υ^{kBr}_{max} cm^{-1}:

1632, 1597, 1563, 1494, 1375, 1361, 1123, 896

Mass, m/z:

$M^{+\cdot}$ 420 (40%), 405 (5%), 377 (15%), 365 (5%).

Acetylation of La-2:

La-2 (50 mg), acetic anhydride (1.5 ml) and pyridine (1.5 ml) were refluxed for 3 hours. After cooling, the mixture was poured on crushed ice and left overnight. The solid was collected, washed with water and dried. On several crystallization from ethylacetate it gave shining colourless needles, m.p. 120-21^{0}C.

Analyed for $C_{31}H_{30}O_9$:

Calcd.: C, 80.13; H, 5.49%

Found: C, 68.45; H, 5.68%

^{1}H-NMR ($CDCl_3$ + DMSO-d_6) on δ scale:

1.45 (s, 6H, chromene methyls), 1.8 (s, 3H), 1.9 (s, 3H) (methyl group on double bond), 3.4 (d, J=7 Hz, benzylic methylene), 2.3 (s, 6H), 2.5 (s, 3H) (acetoxyls), 5.18 (m, 1H, olefinic proton), 5.8 (d, 1H, J=10 Hz, chromene proton), 6.7 (d, 1H, J=10 Hz, chromene protons), 7.4 (m, 2H, aromatic protons), 6.8 (d, 1H, aromatic proton), 7.9 (s, 1H, C_5–H proton).

Methylation of La-2:

La-2 (50 mg), dimethyl sulphate (0.4 ml) anhydrous potassium carbonate (0.5 gm) and dry acetone (15 ml) were refluxed over a water bath for 24 hours. The reaction mixture was filtered and the inorganic residue washed several times with hot acetone. On distilling off the solvent, a brown viscous semi solid mass was left behind. It was treated with hot petroleum ether (60-80^{0}C) to remove unused methyl sulphate. The solid residue on crystallization from chloroform-methyl alcohol gave colourless needles, m.p. 66-68^{0}C.

Analysed for $C_{28}H_{30}O_6$:

Calcd.: C, 72.72; H, 6.49; 3-0Me, 20.13%

Found: C, 72.67; H, 6.44; 1-0Me, 19.5%

La-3:

It was crystallized from methanol as yellow needles, m.p. 236-37^{0}C.

Analysed for $C_{17}H_{14}O_6$:

Calcd: C, 65.0; H, 4.5; OMe, 19.7%

Found: C, 65.23; H, 4.34; OMe, 20.3%

Uv, λ_{max} nm:

EtOH: 268, 334 (inf)

$AlCl_3$: 280

IR, υ^{kBr}_{max} cm^{-1}:

3450 (OH), 1645 (CO) and 1605, 1580, 1515 and 1495 (aromatic).

Mass, m/z:

$M^{+\cdot}$ at 314 (100%), 299 (50.8%), 153 (30.6%), 118 (30.5%)

Acetylation of La-3:

La-3 (50 mg) was dissolved in pyridine (1.5 ml) and acetic anhydride (1.5 ml) and was added to it. The mixture was heated over boiling water bath for 2 hours. On usual work up and crystallization from methanol it gave colourless needles m.p. 182-84^{0}C.

Analysed for $C_{21}H_{18}O_8$:

Calcd: C, 63.31; H, 4.52%

Found: C, 6.25; H, 4.49%

^{1}H-NMR ($CDCl_3$+DMSO) on δ scale:

2.3 (s, 3H, OAc), 2.5 (s, 3H, OAc), 3.8 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 6.8 (s, 1H, C_8 proton), 7.1 (d, 2H, J=9 Hz, 3',5'-H), 7.4 (d, 2H, J=9 Hz, 2',6'–H), 7.8 (s, 1H, H-2).

Methylation of La-3:

La-3 (50 mg), dimethyl sulphate (0.4 ml), anhydrous potassium carbonate (0.5 gm) and dry acetone (15 ml) were refluxed for 24 hours over a water bath. After usual work up a greyish precipitate was obtained. The precipitate was crystallized from chloroform-methanol mixture as colourless shining crystals (30 mg) m.p. 182-84^{0}C. It showed no depression in melting point on mixing with an authentic sample of tectrogenin trimethyl ether.

Analysed for $C_{19}H_{16}O_6$:

Calcd: C, 66.66; H, 5.26

Found: C, 66.42; H, 5.21

La-4:

Fractions obtained from beneze-ethylacetate (1:15) eluate were pooled together, and evaporated to dryness. The yellow solid obtained, on several crystallization from methanol gave light yellow shining needles, m.p. 192-93^{0}C. It gave blue colour with ferric chloride, positive test with boric acid, boric acid in acetic anhydride (dimorth reagent) and negative test with sodium amalgam.

Analysed for $C_{17}H_{14}O_6$:

Calcd.: C, 64.96; H, 4.45; OMe, 19.14%

Found: C, 64.45; H, 4.39; Ome, 19.28%

IR, υ^{KBr}_{max} cm^{-1}:

3520, 1650, 1630, 1602, 1575, 1512, 1450, 1060, 1035, 990, 835.

UV, λ_{max} nm:

EtOH: 270

$AlCl_3$: 280

NaOAc: 277

^{1}H-NMR (60MHz, $CDCl_3$ + DMSO-d_6) on δ scale:

3.8 (3H, s, -OCH_3), 3.9 (3H, s, -OCH_3), 6.5 (1H, s, 8-H), 6.9 (2H, d, J=10 Hz, 3',5'-H), 7.5 (2H, d, J=10 Hz, 2',6'–H), 7.9 (1H, s, 2–H).

Acetylation of La-4:

La-4 (50 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (1 ml) was added to it. The mixture was allowed to stand for 24 hrs at room temperature. After 24 hours crushed ice was added to it and stirred vigorously. The Dirty white solid separated was crystallized from methanol in colourless needles, m.p. 162-63^{0}C.

Analysed for $C_{21}H_{16}O_8$:

Calcd.: C, 63.31; H, 4.52%

Found: C, 63.48; H, 4.59%

Methyl ether:

A mixture of **La-4** (150 mg) freshly distilled dimethyl sulphate (0.7 ml), anhydrous potassium carbonate (1 gm) and dry acetone (20 ml) was refluxed for 36 hours. The reaction mixture was filtered and the inorganic residue washed several times with hot acetone. The washings were combined and concentrated to a smaller volume. To the concentrate, water was added. The dirty white precipitate obtained, was washed with water and dried. On crystallization from ethylacetate colourless plates (130 mg) m.p. 181^{0}C were obtained.

Analysed for $C_{19}H_{18}O_6$:

Calcd.: C, 66.66; H, 5.26%

Found: C, 66.48; H, 5.29%

REFERENCE

1. **'Flora of Tropical Africa'**, by Danil Oliver, Vol. I, 446 (1968).

2. M. Ilyas and Sarwat Sultana, **Phytochemistry, 25,** 963 (1986).

3. Y. Asohania and M. Innbuse, **Ber., 61,** 1646 (1928).

4. L.H. Briggs and R.H. Locker, **J. Chem. Soc.,** 2158 (1949).

5. T.A. Geissman and R.O. Klinton, **J. Amer. Chem. Soc., 68**, 700 (1946).

6. S. Shibata and A. Kasahara, **J. Pharm. Soc., Japan, 72,** 1386 (1952).

7. V. Venkataraman, in **'The Chemistry of Flavanoid Compounds'** (edited by T.A. Geissman), p.72, 70, perganon, New York, 1962).

8. M.L. Wolfrom and B. Wildi, **J. Amer. Chem. Soc., 73,** 235 (1951).

9. J. Shinoda, **J. Pharm. Soc., Japan, 48,** 214 (1928).

10. C.W. Wilson, **J. Amer. Chem. Soc., 61,** 2303 (1939).

11. L. Jurd, **'The Chemistry of Flavanoid Compounds'**, (edited by T.A. Geissman) Perganon Press, Oxford (a) p.107, (1962); (b) p.107-155 perganon New York, (1962); (C) P.8, Perganon press, London (1962).

12. R. Aueja S.K. Mukerjee and T.R. Seshadri, **Tetrahedron, 4,** 256 (1958).

13. B.F. Burrows and W.D. Ollis, **Proc. Chem. Soc., 177** (1960).

14. R.B. Bates, R.H. Carnigham, R.O. Rakutis and J.H. Schaulis, **Chem. & Ind., 40,** 1020 (1962).

15. W.D. Ollis, M.V.J. Ramsay, I.O. Sutherland and S. Mongkuisuk, **Tetrahedron, 21,** 1453 (1965).

16. L.H. Briggs & R.H. Locker, **J. Chem. Soc.,** 3136 (1951).

17. J.W.W. Morgan & R.J. orsler, **Chem & Industry,** 1173 (1967).

18. F.E. King, T.J. King & E. Sellars, **J. Chem. Soc., 563** (1967).

19. T. Swain, **Chem & Ind. (London)**, 1480 (1954).

20. L.H. Briggs & T.P. Cabato, **Tetrahedron, 6,** 145 (1959).

21. L. Prakash, Asifzaman & A.R. Kidwai, **J. Org. Chem., 30,** 3561 (1965).

CHAPTER-IV
VIBURNUM COTINIFOLIUM

Results and

DISCUSSION

CHEMICAL CONSTIUENTS FROM THE LEAVES OF VIBURNUM COTINIFOLIUM (CAPRIFOLIACEAE)

Viburnum is a diverse and adaptable genus of deciduous evergreen shrubs or rarely small trees, distributed chiefly in the North temperate zone, extending as far as Alaska, Labrador, Central and South America and Java, being most abundant in Java, Korea, China and Japan. About seventeen species are found in India.[1a]

The plants of the genus **viburnum** are reputed for their considerable medicinal importance such as antioxidant[1b], antibacterial,[2] astringent, sedative and emmanagogue drug.[3] About seventeen species have been tested for their toxicity.[4] Recently chemotaxonomical investigations in the genus **Viburnum** have been carried out[5] and amentoflavone is considered to be the taxonomic marker in the genus **Viburnum**. While the **Viburnum cotinifolium** is commonly used for the cure of various ailments.[6] Earlier report from this plant is the isolation and characterization of ursolic acid, uvaol[7] and 4,4'-dihydroxy-chalcone-2'-O-(4-O-β-D-glucopyranosyl)-α-L-rhamnopyranoside.[8] The medicinal importance and scanty nature of the work on this plant increased our interest to carryout the comprehensive investigations.

The present discussion deals with the isolation and characterization of the following compounds from the leaves of **Viburnum cotinifolium.**

1. **Eriodictyol**
2. **Luteolin**
3. **Naringenin**
4. **Amentoflavone**
5. **I-5, II-5, I-7, II-7, I-4', II-4'-hexahydroxy [6-O-8] biflavone**
6. **4'-Methoxyscutellarein-6-O-rutinoside**

7. Pectolinarigenin-7-O-rutinoside

8. Scutellarein-7-diglucoside

The leaves of **Viburnum cotinifolium** were collected from Kashmir. They were dried under shade and powdered. The powdered leaves were thoroughly extracted with petrol, benzene and methanol respectively. The petrol and benzene extracts on concentration gave dark green oily mass of fatty nature, therefore were not further examined.

The methanol extract on concentration under reduced pressure gave brown gummy mass. TLC examination of the gummy mass in different solvent systems viz (benzene-pyridine-formic acid (36:9:5), toluene-ethylformate-formic acid (5:4:1) and benzene-methanol-acetic acid (45:8:4) showed it to be a mixture of at least eight major compounds along with some minor ones. The brown gummy mass was chromatographed over silica gel column using successively [benzene, benzene-ethylacatate (9:1-1:1), ethylacetate and ethyl-acetate-methanol (9:1-6:4) as eluting solvents. Appropriate fractions (**ir** spectrum and TLC) were combined.

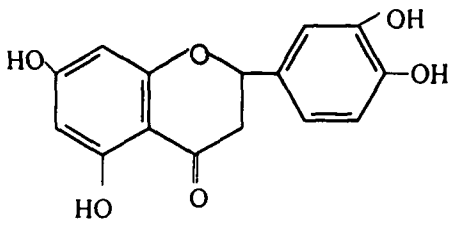
The benzene-ethylacetate (9:1-1:1) eluate was found to be a mixture of five compounds. They were separated by preparative TLC over silica gel plates (BPF, 36:9:5) and labeled as **Vc-1, Vc-2, Vc-3, Vc-4** and **Vc-5**. The fractions obtained from the column by EtOAc, EtOAc-MeOH (9:1,8:2) were found to be mixtures of two compounds, having the same R_f values they were pooled together and separated by TLC using EtOAc-EtMeCO-AcOH-H_2O (5:3:1:1) as solvent system. The homogeneity of the fractions was further checked by paper chromatography using n-BuOH-AcOH-H_2O (4:1:5) as developing solvent, they were marked as **Vc-6** and **Vc-7**. The fractions obtained from the column by EtOAc-MeOH (7:3) gave a single compound along with some minor impurities, which were removed by repeated crystallization with methanol-benzene, the compound so obtained was labeled as **Vc-8**.

Vc-1:

It was crystallized with $CHCl_3$-MeOH as yellow cubes m.p. 263°C, Elemental analysis agreed to the molecular formula $C_{15}H_{12}O_6$. The colour reaction and positive Shinoda's test[9], coupled with the appearance of two protons multiplet (C_3-2H) centered at δ 3.0 and a proton multiplet (C_2-H) at δ 5.40 in the **^{1}H-nmr** spectrum confirmed the presence of a flavanone nucleus. A pair of meta-coupled doublets at δ 6.66 and δ 6.88 for one proton each indicate the presence of H-6 and H-8 protons and a multiplet of three protons at δ 7.28 corresponding to H-2',5',6'.**IR** spectrum revealed the presence of a phenolic OH (υ^{KBr}_{max} 3430 cm^{-1}) and a conjugated >C=O group (1680 cm^{-1}).

Acetylation of **Vc-1** with Ac_2O/py gave a tetraacetate derivative m.p. 143-44°C. It was found to be identical with the authentic sample of eriodictyol tetraacetate (R_f value, m.p., m.m.p., characteristic shade in **uv** light).

One the basis of the above results, the **Vc-1** was characterized as **5,7, 3',4'-tetrahydroxy flavanone (eriodictyol)[10] (I).**



(I)

Vc-2:

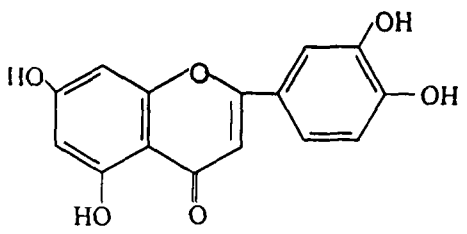
It was crystallized with ethylacetate-acetone as yellow crystals, m.p. >320°C. Elemental analysis agreed with the molecular formula $C_{15}H_{10}O_6$. It gave greenish brown colour with ferric chloride and responded positively to Shinoda's test[9]. The **uv** spectrum showed λ^{MeOH}_{max} at 256, 265 and 345 nm. **IR** spectrum revealed the presence of phenolic OH (3400 cm^{-1}), α,β-unsaturated >C=O group (1640 cm^{-1}) and aromatic nucleus (800-840 cm^{-1}). The changes in

the presence of diagnostic shift in its **uv** spectrum pointed out the presence of free hydroxyl groups at 5,7,3' and 4'-position.

Acetylation of **Vc-2 (Table-1)** gave a tetraacetate (Vc-2Ac), m.p.≈ 200^{0}C. The **^{1}H-nmr** spectrum of Vc-2Ac evidenced the presence of four aromatic acetoxyls integrated for 12 protons at δ 2.43, δ 2.35 and δ 2.33 assigned to OAc-5, OAc-7 and OAc-3',4'. The **^{1}H-nmr** spectrum also established a disubstituted ring-B as it showed a typical multiplet at δ 7.76 (J_1 = 8 Hz, J_2=2.20 Hz)[4-6] and δ 7.70 (J_1=2.20 Hz, H-2'). Another ortho-coupled doublet integrating for one proton was observed at δ 7.38 (J=8.0 Hz, H-5'). This could be attributed to 3',4'-substitution of ring-B. The 5,7-disubstitution of the ring-A is demonstrated by two meta-coupled doublets at δ 6.85 and δ 7.35 (J=2.0 Hz, each) assigned to H-6 and H-8 protons which have shifted slightly downfield due to derivatization. A sharp singlet at δ 6.61 is assigned to H-3 proton.

The **mass** spectrum of Vc-2Ac is fully in agreement with structure (**II**). It showed the molecular ion peak at m/z 454 in accordance with a flavone containing four acetoxyl groups. The subsequent removal of four acetoxyls gave fragments at m/z 412, 370, 328 and 286. The fragment at m/z 286 is observed as the base peak. A $[B_1]^{+\cdot}$ fragment at m/z 134 fully supported a ring-B with two hydroxyl groups. An $[A_1+H]^+$ fragment at m/z 153 is consistent with the ring-A having two OH-groups, **(Scheme-I)**

On the basis of above results, **Vc-2** is characterized as **5,7,3',4'-tetrahydroxy flavone (luteolin)[11] (II).**



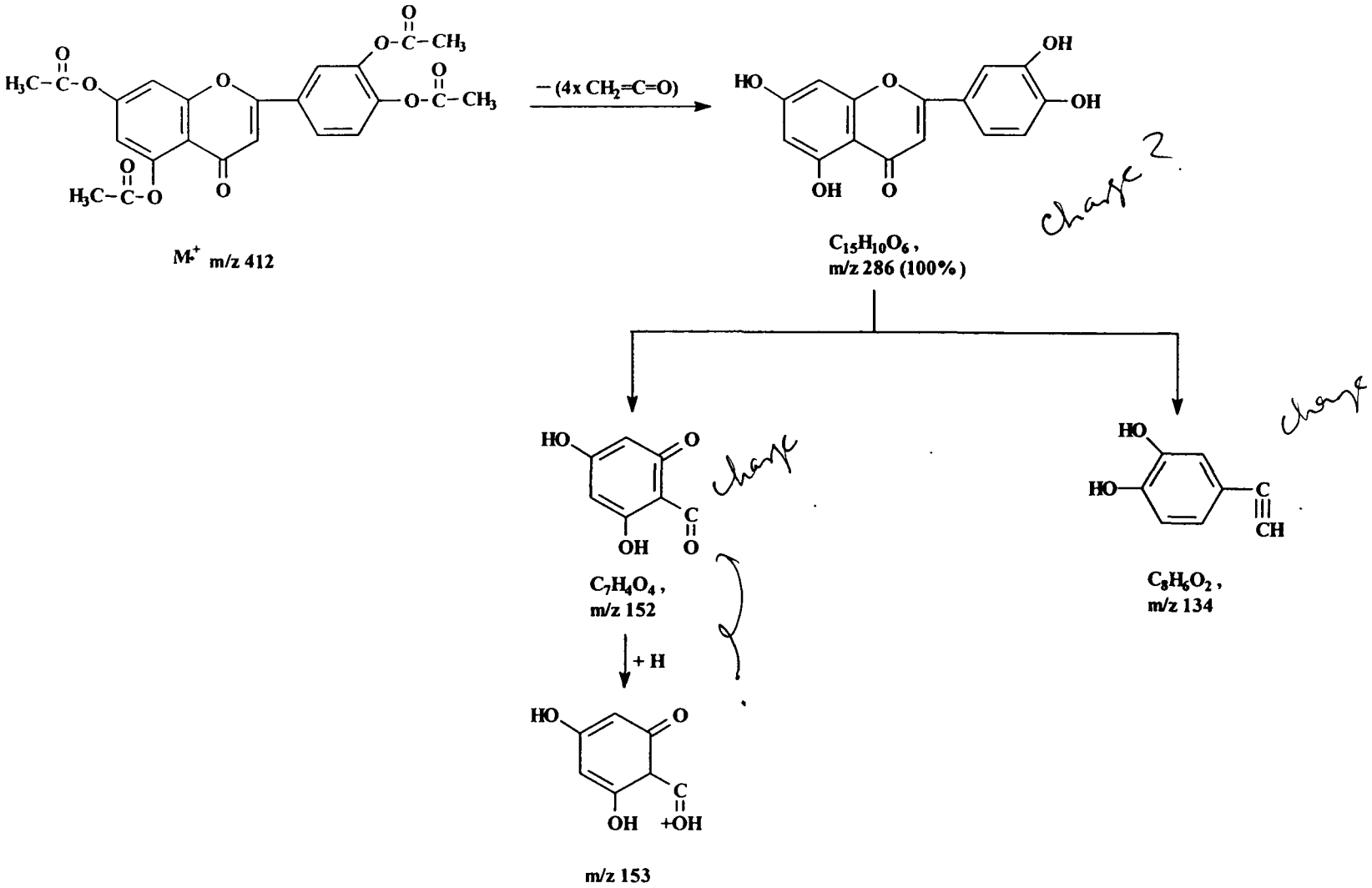
(II)

Table-1

^{1}H-NMR spectral data of Vc-2

Assignments	No. of Protons	Signal
H-6	1	6.85 (d, J=2.0 Hz)
H-8	1	7.35 (d, J=2.0 Hz)
H-3	1	6.61 (s)
H-5'	1	7.38 (d, J=8.0 Hz)
H-6'	1	7.76 (dd, J_1=8.0 Hz, J_2=2.20 Hz)
H-2'	1	7.70 (d, J=2.20 Hz)
OAc-5	3	2.43 (s)
OAc-7	3	2.35 (s)
OAc-3',4'	6	2.33 (s)

s=singlet, d= doublet, spectrum run in $CDCl_3$ at 300 MHz using TMS as internal standard (δ-scale).

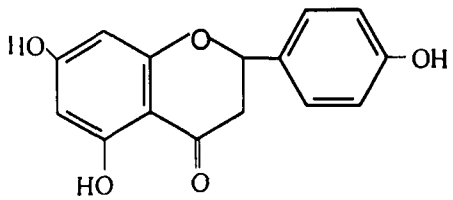

− (4x CH2=C=O)
M+ m/z 412
C15H10O6 ,
m/z 286 (100%)
C7H4O4 ,
m/z 152
+ H
m/z 153
C8H6O2 ,
m/z 134

(Scheme -I)

Vc-3:

Crystallized from benzene-acetone as yellow needles m.p. 248-50^{0}C. It was characterized as **naringenin (III)** by direct comparison with an authentic sample of naringenin (R_f value, m.p., m.mp, co-chromatography and **^{1}H-nmr**). The result of **^{1}H-nmr** is recorded in **(Table-2).**

Vc-3 was therefore, assigned the structure **5,7,4'-trihydroxy flavanone (naringenin)[12] (III).**



(III)

Table-2

^{1}H-NMR spectral data of Vc-3

Assignments	No. of Protons	Signal
H-2', 6'	2	7.40 (d, J=8.5 Hz)
H-3', 5'	2	6.94 (d, J=8.5 Hz)
H-6	1	6.64 (d, J=2.5 Hz)
H-8	1	6.86 (d, J=2.5 Hz)
H-2	1	5.20 (q, J_1=12 Hz, J_2=4 Hz)
H-3,3	2	2.79-2.98 (m, J_1=12 Hz, J_2=4 Hz, J_3=17 Hz)

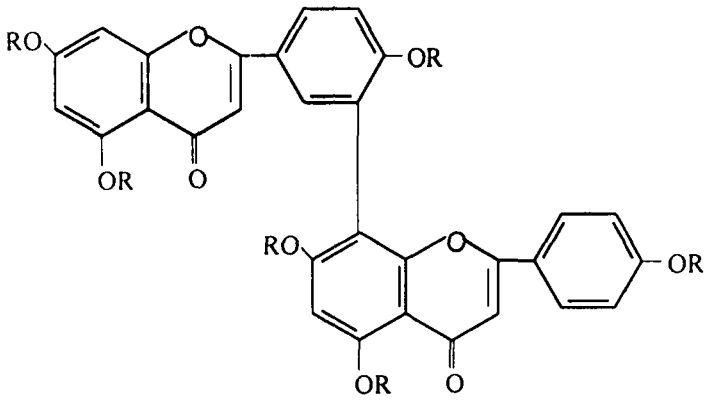
d=doublet, q=quartet, m=multiplet, spectrum run in $(CD_3)_2$ CO at 300 MHz using TMS as internal standard (δ- scale).

Vc-4:

It was crystallized from methanol as yellow needles, m.p. 254-55^{0}C. The elemental analysis as well as molecular ion peak at m/z 538 led to its molecular formula as $C_{30}H_{18}O_{10}$. A pink colour with Zn/HCl suggested it to be a flavonoid.[10] It gave a positive colour with $FeCl_3$. Derivatization of the compound gave a methylether (Vc-4Me) (IV-b), m.p. 226-27^{0}C and acetate (Vc-4Ac) m.p. 240-42^{0}C. The **^{1}H-nmr** spectrum of Vc-4Me showed the presence of six singlets at δ 4.05, 3.92, 3.90, 3.82, 3.76 and 3.72 integrating for three protons each, assigned to six methoxyl groups. In the aromatic region, it showed two singlets at δ 6.50 and δ 6.57 for one proton each, characteristic of I-3 and II-3 protons respectively. A singlet at δ 6.63 was assigned to H-II-6. The spectrum **(Table-3)** showed clearly ABX and A_2B_2 systems associated with ring I-B and II-B, substituted at I-3',4' and II-4', respectively. A pair of doublets at δ 6.73 and δ 7.38 (J=9 Hz) of two protons each for II-2',6' and II-3',5' and a quartet of one proton at δ 7.94 (J_1=9 Hz, J_2=2.5 Hz) for I-6' and a doublet at δ 7.85 (J=2.5 Hz) of one proton was for I-2'. Thus ring I-B and II-A of the biflavone, seemed to be involved in interflavonoid linkage. In particular the value showed that I-3' is linked to either II-6 or II-8. The observations that in biflavones, having aromatic substituents at I-8, the I-5-OMe group generally appears below δ 4.00 **(Table-4)** led to believe that substituent (flavone unit) in Vc-4Me was located at II-8 and not at II-6. Further the signals for all methoxyl groups, on change of solvent for $CDCl_3$ to benzene moved up-field, showing that every methoxyl group had at least one ortho proton, therefore a II-8, rather II-6 linkage was confirmed.

The structure was further supported by **mass** fragmentation (IV-C) and by comparing the **^{1}H-nmr** spectrum of its acetate (Vc-4Ac) with that of authentic sample of amentoflavone hexaacetate **(Table-5)**. The **^{1}H-nmr** spectrum of the acetate showed six acetoxyl groups integrating for 18 protons.

Vc-4, was thus assigned the structure, **1-4', II-4', I-5, II-5, I-7, II-7 hexahydroxy [I-3', II-8] biflavone (amentoflavone)** [14,15,16] **(IVa).**



(IV)

(a) R = H
(b) R = Me
(c) R = Ac

Table-3

^{1}H-NMR spectral data of Vc-4Me

Assignments	No. of Protons	Signal
H-I-8	1	6.46 (d, J=2.45 Hz)
H-I-6	1	6.32 (d, J=2.5 Hz)
H-II-6	1	6.63 (s)
H-I-6'	1	7.94 (q, J_1=9.0 Hz, J_2=2.5 Hz
H-I-2'	1	7.85 (d, J=2.5 Hz)
H-I-5'	1	7.10 (d, J=9.0 Hz,)
H-II-2',6'	2	7.38 (d, J=9.0 Hz)
H-II-3',5'	2	6.73 (d, J=9.0 Hz)
H-I-3	1	6.50 (s)
H-II-3	1	6.57 (s)
OMe-I-5	3	3.92 (s)
OMe-II-5	3	4.05 (s)
OMe-I-7	3	3.90 (s)
OMe-II-7	3	3.82 (s)
OMe-I-4'	3	3.76 (s)
OMe-II-4'	3	3.72 (s)

s= singlet, d=doublet, q= quartet. Spectrum run in $CDCl_3$, at 300 MHz, using TMS as internal standard (δ-scale).

Table-4

S.No.	Biflavonoids	I-5-OMe (δ)	II-5-OMe (δ)
1.	Cupressuflavone-hexamethyl ether	4.12	4.12
2.	Amentoflavone-hexamethyl ether	3.87	4.06
3.	Agathisflavone-hexamethyl ether	3.59	4.05
4.	Hinokiflavone-pentamethyl ether (I-4'-O-II-8)	4.00	4.08
5.	Vc-4Me	3.92	4.05

Table-5

^{1}H-NMR spectral data of Vc-4Ac and amentoflavone hexacetate

Assignment	Vc-4Ac (δ)	Amentoflavone hexacetate
H-I-8	7.26 (d, 1H, J=3 Hz)	7.27 (d, 1H, J=3 Hz)
H-I-6	6.84 (d, 1H, J=3 Hz)	6.67 (d, 1H, J=3 Hz)
H-II-6	7.01 (s, 1H)	7.03 (s, 1H)
H-I-6'	7.98 (q, 1H, J_1=8 Hz, J_2 = 3 Hz)	8.01 (q, 1H, J_1=8 Hz, J_2 = 3 Hz)
H-I-2'	8.03 (d, 1H, J=3 Hz)	8.06 (d, 1H, J=3 Hz)
H-I-5'	7.46 (d, 1H, J=9 Hz)	7.52 (d, 1H, J=9 Hz)
H-II-3',5'	7.06 (d, 2H, J=9 Hz)	7.08 (d, 2H, J=9 Hz)
H-I-3, II-3	6.68, 6.65 (s, 2H)	6.70, 6.68 (s, 2H)
OAc-I-4', II-4'	2.28, 2.23 (s, 6H)	3.33, 3.38 (s, 6H)
OAc-I-7, II-7	2.05, 2.01 (s, 6H)	2.11, 2.06 (s, 6H)
OAc-I-5, II-5	2.45, 2.41 (s, 6H)	2.50, 2.41 (s, 6H)

s=singlet, d=doublet, q=quartet spectrum run in $CDCl_3$ at 100 MHz, using TMS as internal standard (δ-scale)

Vc-5:

It was crystallized with chloroform-methanol as pale yellow grannular crystals, m.p. 222-23 °C. It gave pink colour with Zn/HCl and yellow to orange colour with Mg/HCl, suggested it to be a flavone[17]. Elemental analysis and the molecular ion peak at m/z 554 led to its molecular formula as $C_{30}H_{18}O_{11}$. The **uv** spectrum showed the λ_{max} at 270 and 336 nm. Analysis with classical shift reagents[18], $AlCl_3$, NaOAc and NaOMe exhibited the presence of free hydroxyls at 5,7 and 4′- positions corresponded to apigenin. The molecular extinction coefficient of **Vc-5** was found to be approximately double as compared to the corresponding monomer pointing out it to be a biflavonoid structure.

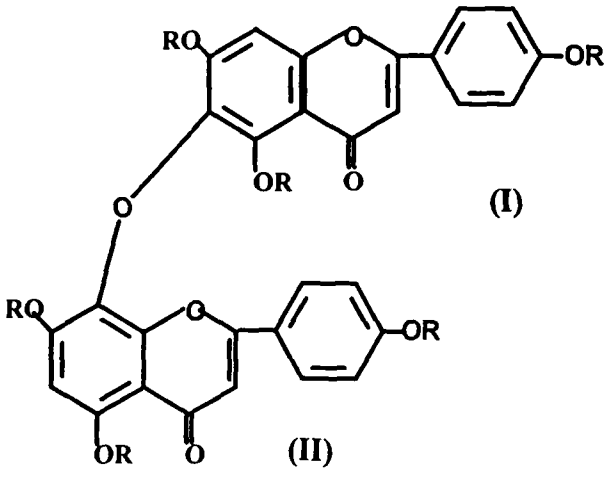
The **^{1}H-nmr** spectrum **(Fig.-I, Table-6)** showed it to be an unsymmetrical biflavonoid as it showed two pairs of ortho-coupled doublets integrating for two protons each. Two doublets centered at δ 6.63 (J=8.5 Hz) and δ 7.60 (J=8.5 Hz), were assigned to H-1-3′,5′ and H-I-2′,6′ the other pair resonated at δ 7.02 (J=8.5 Hz) and δ 7.90 (J=8.5 Hz), ascribed to H-II-3′,5′ and H-II-2′,6′ respectively. Two independent singlet at δ 6.78 and 6.80 were attributed to H-II-6 and H-I-8 protons. The remaining H-I-3 and H-II-3 protons were indicated by two singlets of one proton each at δ 6.19 and 6.38.

Methylation of **Vc-5** afforded hexamethyl ether **(V-b)** m. p. 150°C which showed bright yellow shade under **uv** light. The shade and R_f value of its hexamethyl ether were not comparable with the characteristic shades[19a,b] and R_f values of authentic samples of hexamethyl ether of cupressuflavone [I-8,II-8], amentoflavone [I-3′, II-8], agathisflavone [I-6, II-8], hinokiflavone [I-4′-O-II-6] and robustaflavone [I-3′,II-6], thus ruled out the possibility of its being the above biflvone. The possibility of succedanea flavone [I-6,II-6][20] was also not feaseable as the **^{1}H-nmr** (**Table-7**) of **Vc-5** showed unsymmetrical nature of the biflavone. The mode of interflavonoid linkage was further confirmed by benzene induced shift method.

Vc-5 hexamethyl ether, on change of solvent from d_1-chlroroform to d_6-benzene, only five of six methoxy groups showed large upfield shifts (**Table-8**) showing that every methoxy group has at least one proton at ortho position, one methoxy group was unique in that upto 50% dilution **(Table-8)** with benzene no shift was seen and then strong downfield shift was evidenced. It was reasonable to assume that the methoxy group in question was the one at C-5, flanked by ring II-A on one side and a carbonyl group on the other. This results showed that the linkage must be [I-6-O-II-8] for **Vc-5** hexamethyl ether. The above observation and mass spectrum, discussed below, of **Vc-5** suggested it to be 6-O-8 biflavone. Acetylation of **Vc-5** with Ac_2O/py gave hexaacetate **(V-c), (Table –9).**

The **mass** spectrum **(Fig.-II)** showed the molecular ion peak at m/z 554. The fragment ions at m/z 269, 285, 318, 326, 419 proved the inter flavonoidic likage as 6-0-8. The other fragments are rationalized from the **(Scheme-II)**

On the basis of above data, the compound **Vc-5**, is characterized as **I-5, II-5, I-7, II-7, I-4', II-4'-hexahydroxy[6-O-8] biflavone (V-a)**, being reported for the first time.



(V)

(a) R = H
(b) R = Me
(c) R = Ac

Table-6

^{1}H-NMR spectral data of Vc-5

Assignment	Chemical shift
H-I-3	6.19 (1H, s)
H-II-3	6.38 (1H, s)
H-II-6	6.78 (1H, s)
H-I-8	6.80 (1H, s)
H-I-3′,5′	6.63 (2H, d, J=8.5 Hz & 2.0 Hz)
H-I-2′,6′	7.6 (2H, d, J=8.5 Hz & 2.0 Hz)
H-II-3′,5′	7.02 (2H, d, J = 8.5 Hz & 2.0 Hz)
H-II-2′,6′	7.90 (2H, d, J=8.5 Hz & 2.0 Hz)

s = singlet, d = doublet, spectrum in DMSO-d_6 at 400 MHz using TMS as internal standard on (δ-scale).

Table-7

^{1}H-NMR spectral data of Vc-5Me

Assignment	Chemical shift
OMe-II-5	4.00 (3H, s)
OMe-I-5	3.88 (3H, s)
OMe-I-7	3.86 (3H, s)
OMe-II-7	3.76 (3H, s)
OMe-I-4′	3.74 (3H, s)
OMe-II-4′	3.59 (3H, s)
H-I-3	6.21 (1H, s)
H-II-3	6.40 (1H, s)
H-II-6	6.77 (1H, s)
H-I-8	6.82 (1H, s)
H-I-3′,5′	6.66 (2H, d, J=8.5 Hz & 2.0 Hz)
H-I-2′,6′	7.61 (2H, d, J=8.5 Hz & 2.0 Hz)
H-II-3′,5′	7.06 (2H, d, J=8.5 Hz & 2.0 Hz)
H-II-2′,6′	7.92 (2H, d, J=8.5 Hz & 2.0 Hz)

s = singlet, d = doublet, spectrum run in $CDCl_3$ at 300 MHz using TMS as internal standard on (δ-scale).

Table-8

Shift of methoxy resonances of Vc-5Me

Signal in $CDCl_3$ (Hz)	Signal in C_6H_6 (Hz)	Shift (Hz)
405	358	+47
390	330	+60
389	335	+54
380	326	+54
375	305	+70
362	385	–23

Table-9

^{1}H-NMR spectral data of Vc-5Ac

Assignment	Chemical shift
OAc-I-5, II-5	2.45 (6H,s)
OAc-I-5, II-7	2.35 (6H, s)
OAc-I-4, II-4	2.33 (6H, s)
H-I-3	6.23 (1H, s)
H-II-3	6.45 (1H, s)
H-II-6	6.79 (1H, s)
H-I-8	6.85 (1H, s)
H-I-3′,5	6.70 (2H, d, J=8.5 Hz & 2.0 Hz)
H-I-2′,6′	7.69 (2H, d, J=8.5 Hz & 2.0 Hz)
H-II-3′,5′	7.08 (2H, d, J=8.5 Hz & 2.0 Hz)
H-II-2′,6′	7.95 (2H, d, J=8.5 Hz & 2.0 Hz)

s = singlet, d = doublet, spectrum in $CDCl_3$ using TMS as internal standard on (δ-scale).

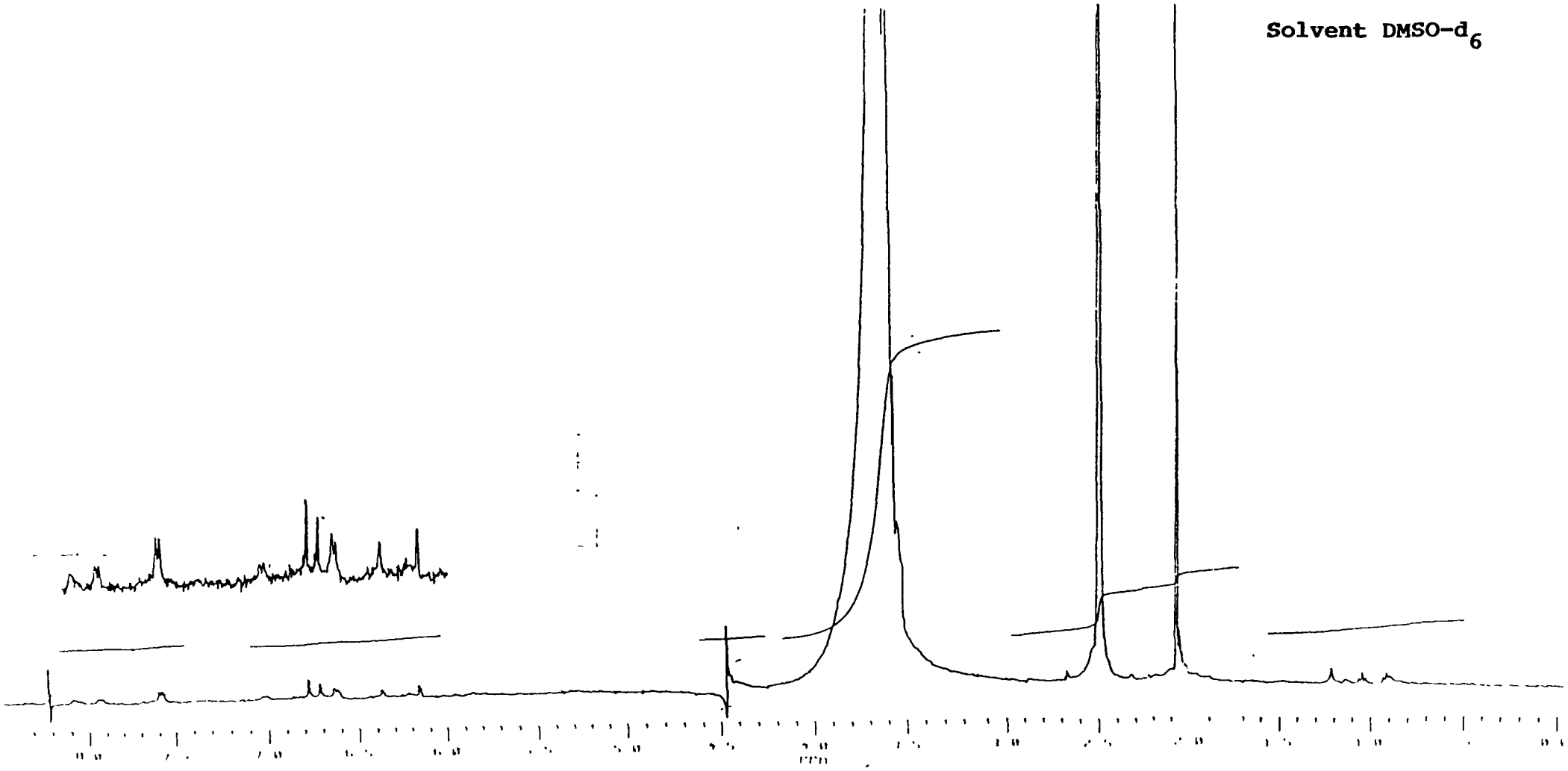


Fig.-I

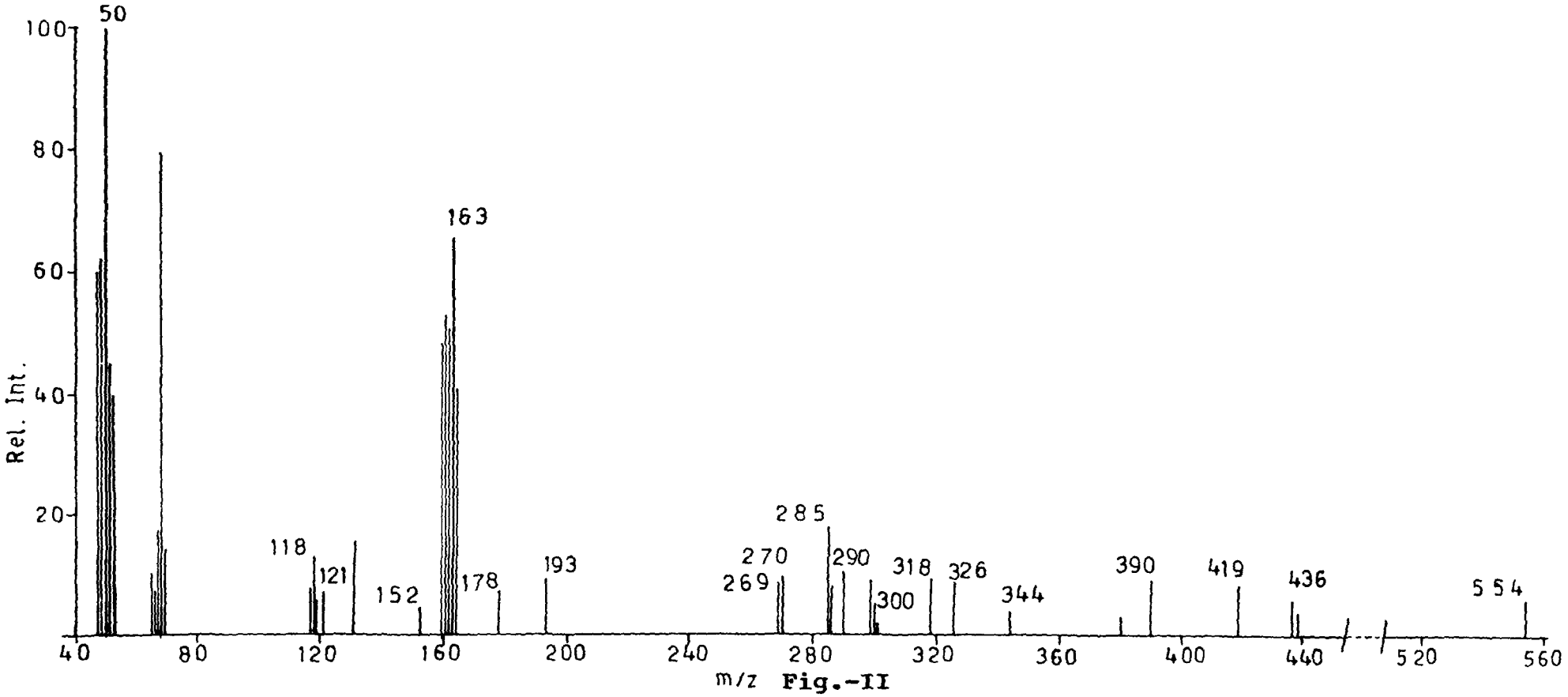
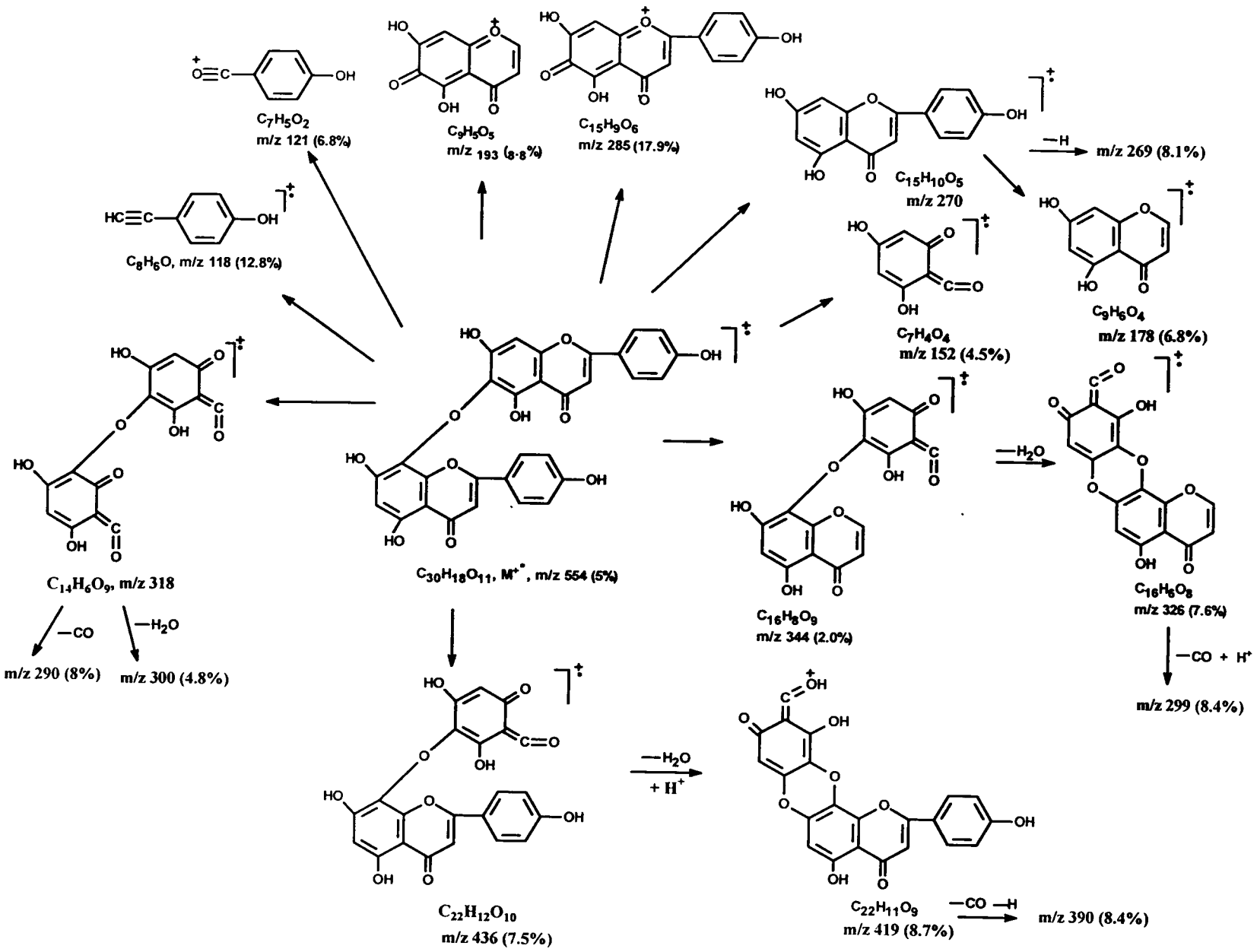

Rel. Int.
100
80
60
40
20
50
163
118
121
152
178
193
269
270
285
290
300
318
326
344
390
419
436
554
40
80
120
160
200
240
280
320
360
400
440
520
560
m/z

Fig.-II

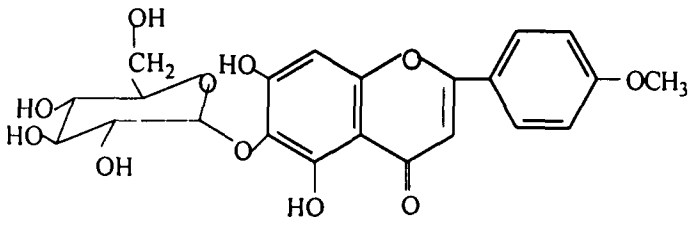

C7H5O2
m/z 121 (6.8%)
C9H5O5
m/z 193 (8.8%)
C15H9O6
m/z 285 (17.9%)
C15H10O5
m/z 270
−H
m/z 269 (8.1%)
C8H6O, m/z 118 (12.8%)
C7H4O4
m/z 152 (4.5%)
C9H6O4
m/z 178 (6.8%)
C14H6O9, m/z 318
C30H18O11, M+•, m/z 554 (5%)
C16H8O9
m/z 344 (2.0%)
−H2O
C16H6O8
m/z 326 (7.6%)
−CO
−H2O
m/z 290 (8%)
m/z 300 (4.8%)
−CO + H+
m/z 299 (8.4%)
C22H12O10
m/z 436 (7.5%)
−H2O
+ H+
C22H11O9
m/z 419 (8.7%)
−CO −H
m/z 390 (8.4%)

(Scheme II)

Vc-6:

The compound **Vc-6** on crystallization with ethylacetate-alcohol mixture gave pale yellow needles. It did not melt upto 275^{0}C. Elemental analysis agreed to the molecular formula $C_{28}H_{32}O_{15}$. Positive Shinoda's test and **ultra-violet** spectrum showed it to be a flavone. The **infrared** spectrum displayed strong bands at 3450 cm^{-1} (OH) and 1650 cm^{-1} (C=O). The **ultra-violet** spectrum showed λ_{max} in methanol at 290 nm and 334 nm. Shifts of λ_{max} in the presence of classical reagents[18,20] indicated the presence of free hydroxyls at 5 and 7-positions of the flavone glycoside and a solitary methoxyl group at 4'-position.

Total acid hydrolysis of the glycoside gave an aglycone, glucose and rhamnose. The sugars were identified by paper co-chromatography with authentic samples of sugars. The aglycone was characterized as 4'-methoxy scutellarein (VI-a) m.p. 268.70^{0}C by spectral and chromatographic comparison with an authentic sample.[21] Partial hydrolysis of **Vc-6** was accomplished by heating it with 1% H_2SO_4 over boiling water bath for 1hour. The sugar was identified as rhamnose. The glycoside (VII) obtained on further hydrolysis (acidic and enzymatic) afforded 4'-methoxy scutellarein (VI-a) and glucose.



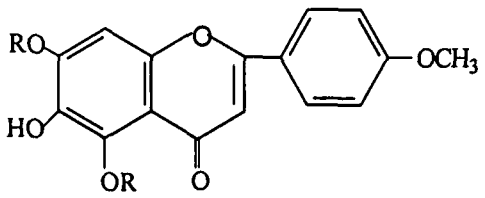
(VII)

The quantitative estimation of sugars by somogyi's copper micro method[22] indicated the presence of 2 mole of sugars per mole of aglycone.

Acetylation of **Vc-6** with acetic anhydride and pyridine gave an acetate m.p. 166-68^{0}C.

The **^{1}H-nmr** spectrum **(Fig.-III)** of the acetate showed A_2B_2 pattern for ring-B protons. A doublet at δ 8.07 (J=9 Hz) was assigned to 2',6' protons and a doublet at δ 7.40 (J=9 Hz) for 3',5' protons. Two singlets for one proton each at δ 6.87 and δ 6.65 were assigned to the C-8 and C-3 protons respectively. A signal at δ 3.79 (3H, s) indicated the presence of one methoxyl group. A total of 24 protons were observed over the range of δ 2.09-2.48, attributed to 8 acetoxyl groups. The signals over the range δ 3.40-5.44 accounted for fifteen protons of gluco-rhamnose residue. The position of H-1 proton of rhamnosyl at δ 4.42 (d) and glucosyl at δ 5.14 (d) along with the integration of the region at δ 4.42-5.44 and δ 3.44-4.40 (ratio.8:4) fully supported the 6-O-rutinosyl group.[18,22]

Spectral data thus, suggested that a rutinosyl group is attached to 6-position of the aglycone and this was further confirmed by the hydrolysis of the methylated glycoside. The partial methyl ether was characterised as 6-hydroxy, 4',5,7-trimethoxy flavone (VI-b) by its spectral studies. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-glucose by SiO_2-TLC and paper chromatography according to Petek[24].

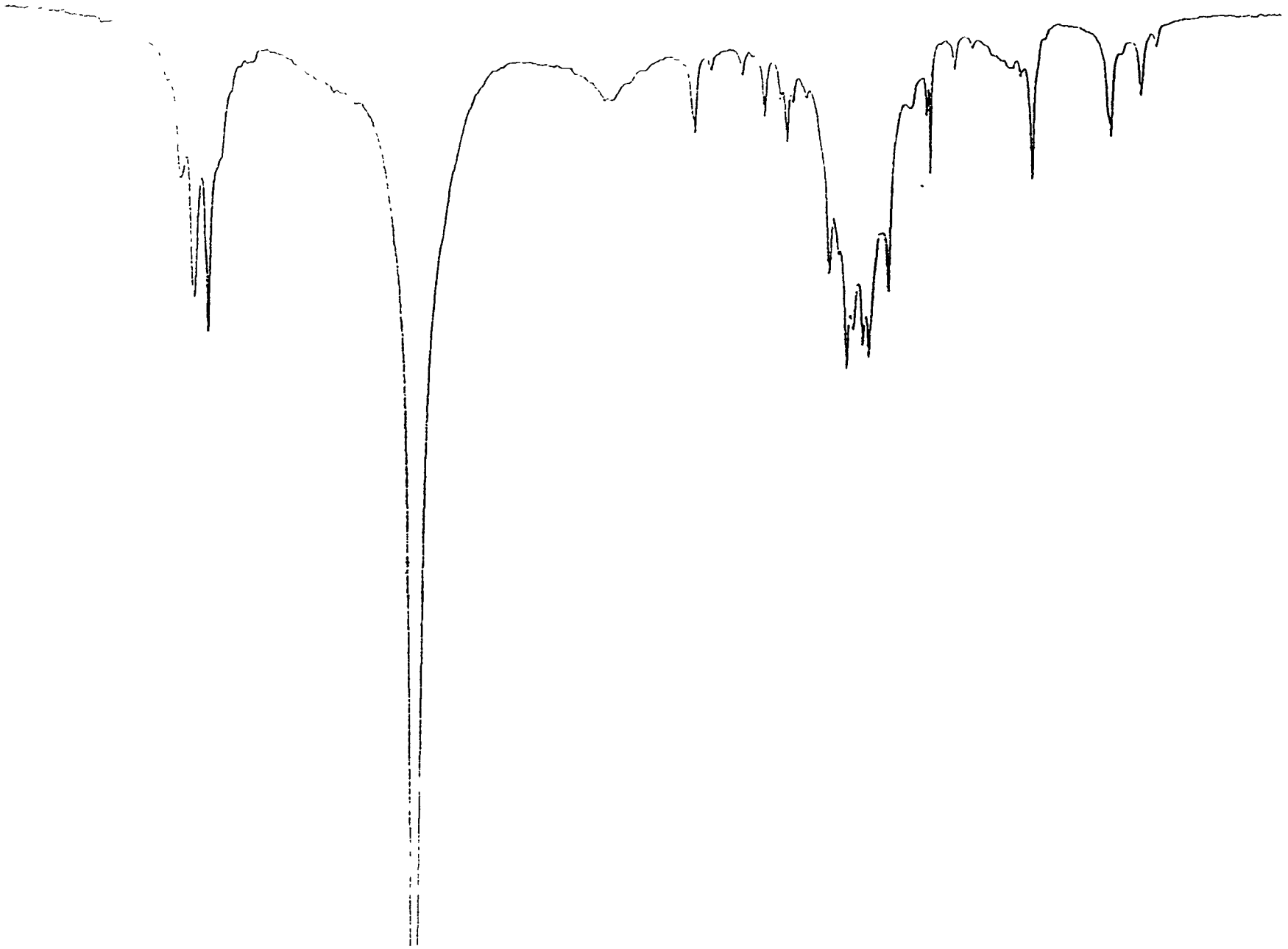


(VI)

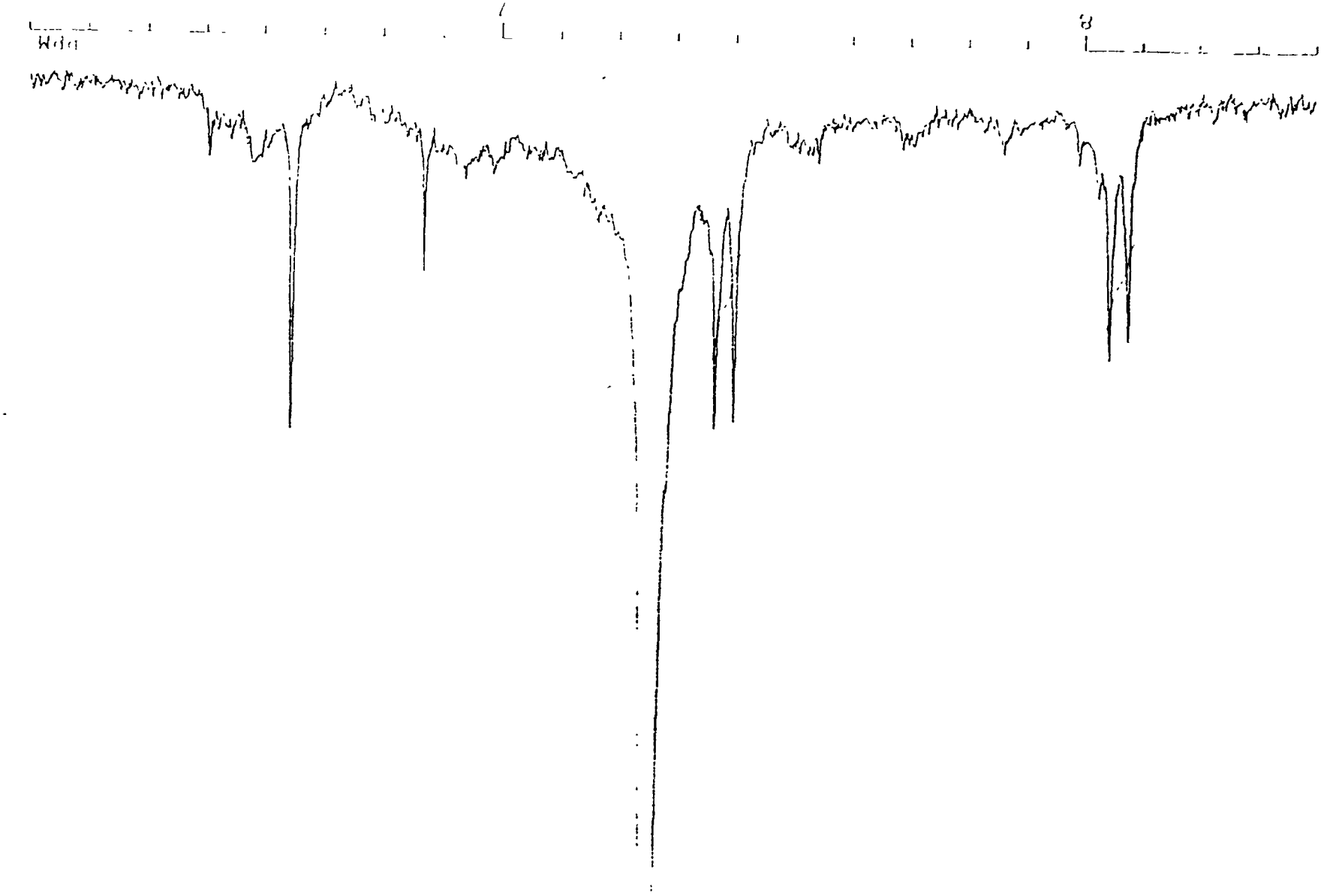
(a) : R = H
(b) : R = CH_3

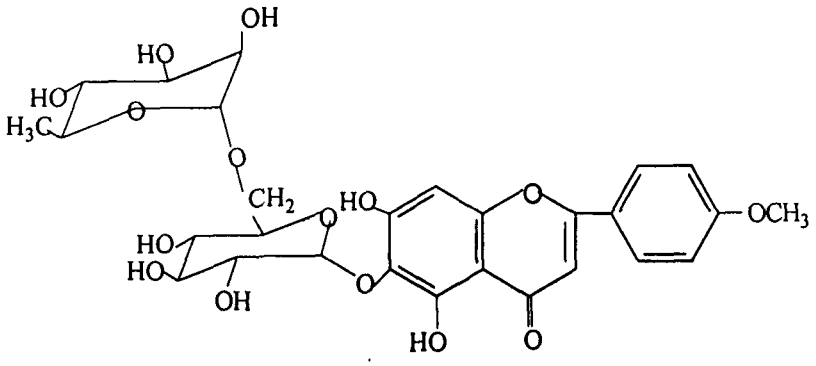
On the basis of these results, the flavone glycoside (Vc-6) was identified as **4'-methoxy scutellarein 6-O-rutinoside (VIII).**

Fig.-III



PPM
3
4
5
6





(VIII)

Vc-7:

The fraction **(Vc-7)** on crystallization with ethylacetate-methanol mixture gave yellow needles, m.p. 248-50^{0}C. Elemental analysis agreed to the molecular formula $C_{29}H_{34}O_{15}$. The glycosidic nature of the compound was evidenced by the positive Molisch test obtained after hydrolysis, and by the – **^{1}H-nmr** spectrum of its acetate. It gave green colour with alcoholic $FeCl_3$ and pink colour on treatment with Zn / HCl. The **ultra-violet** spectrum showed λ_{max} at 276 and 335 nm. A red shift of long wavelength to 350 nm with $AlCl_3$/HCl indicated the presence of a free 5-hydroxyl group.

The **infra-red** spectrum of the compound **Vc-7** showed a carbonyl band at 1650 cm^{-1} and a strong absorption at 840 cm^{-1} indicated a paradisubstututed aromatic ring.

Hydrolysis of **Vc-7** with 10% HCl yielded glucose, rhamnose and an aglycone, m.p. 214-16^{0}C. The **uv** spectrum of the aglycone showed a red shift of 18 nm in band II with NaOAc (absent in glycoside) indicating thereby the attachment of sugar/s at position 7 of the aglycone. The aglycone was characterized as pectoliuarigenin (IX-a) by its melting and mixed melting points and co-TLC with an authentic sample. Further confirmation to its identity was furnished by its derivitization and spectral studies.

The aglycone was acetylated by heating it with acetic anhydride and pyridine over a boiling water bath. The diacetate obtained on crystallization

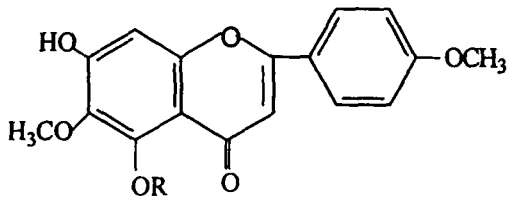
from ethanol gave white needles, m.p. 110-12^{0}C. The **^{1}H-nmr** spectrum of the acetate **(Table-10)** showed two acetoxyl signals at δ 2.41 and δ 2.52 and two methoxyl singlets at δ 3.88 and δ 3.99. A sharp singlet at δ 6.50 was assigned to C-3 proton. A signlet at δ 7.01 for one proton can be assigned to an aromatic proton shielded by two ortho and one para oxygen. It can arise from the C-6 proton of a 5,7,8-trioxygenated flavone or C-8 proton of a 5,6,7-trioxygenated flavone. It was assigned to C-8 proton as it appeared more downfield from the region of a C-6 proton. The other aromatic protons appeared as two doublets at δ 6.85 (J=9 Hz) and δ 7.60 (J=9 Hz), characteristic of a para disubstituted benzene ring.

On the basis of **uv** spectral evidences[25] and formation of diacetate, the two methoxyl groups are therefore placed at C-6 and C-4' position of the aglycone.

The glycoside on acetylation formed a crystalline acetate m.p. 136-38^{0}C. The **^{1}H-nmr** spectrum of the acetate **(Table-11)** indicated it to be rutinoside as it showed six alcoholic acetoxyls at δ 1.90 (3H) and δ 2.12 (15 H) and one aromatic acetoxyl at δ 2.50 (3H). The position of anomeric protons of rhamnosyl and glucosyl moieties (H-1‴, H-1″) at δ 4.54 (1H, d, J=2.5 Hz) and at δ 5.30 (1H, d, J=8 Hz) indicated the presence of α-L Rhamunose and β-D-glucose. The rhamnosyl methyl at δ 0.88 (d, J=6 HZ) along with the integration of the region δ 4.42-5.50 and δ 3.40-4.40 (ratio 8:4) of the glycoside acetate was in full agreement with 7-O-rutinosyl group.[26] Two sharp singlets at δ 3.91 and δ 3.96 were assigned to two methoxyl groups. The lone aromatic proton of ring-A appeared as a signlet at δ 7.00. The C-3 proton was observed as a sharp singlet at δ 6.62. A_2B_2 pattern of the ring-B was evidenced by two doublets at δ 7.75 (J=9 Hz) and δ 7.10 (J=9 Hz). The two doublets were assigned to 2',6' and 3',5' protons respectively.

The position of the sugar/s residue in the glycoside was confirmed by the hydrolysis of the methylated glycoside. Partially methylated aglycone (IXb)

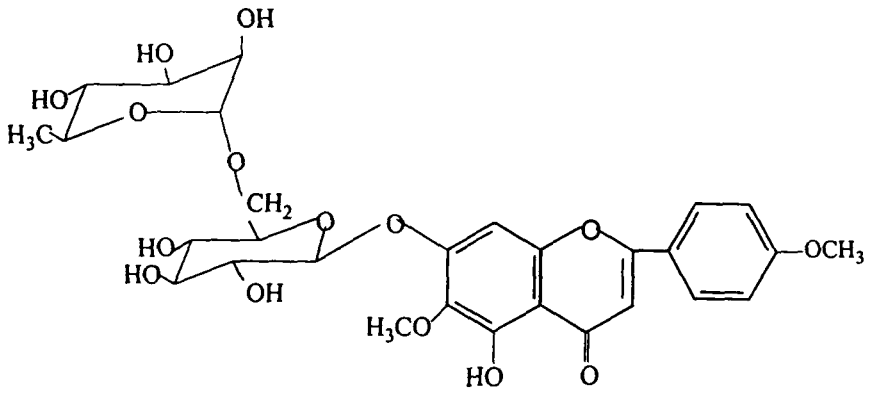
corresponded to molecular formula $C_{18}H_{16}O_6$ and showed a red shift of 16 nm in band II with NaOAc, indicating free C-7 hydroxyl group. The formation of this partial methyl ether formed, thus proved that both the sugars were linked to C-7 hydroxyl of the aglycone. The methylated sugars were identified as 3,4,6-tri-O-methyl-rhamnose and 2,3,4,tri-O-methyl-D-glucose, thus confirming inter sugar linkage as rutinoside (1→6).



(IX)

(a) : R = H
(b) : R = CH_3

On the basis of above discussion **Vc-7** was characterized as **pectolinarigenin 7-O-rutinoside (pectolinarin) (X).**



(X)

Table-10

^{1}H-NMR spectral data of acetate of Vc-7 aglycone

Assignment	No. of Protons	Signals
H-8	1	7.01 (s)
H-3	1	6.50 (s)
H-3',5'	2	6.85 (d, J=9 Hz)
H-2',6'	2	7.60 (d, J=9 Hz)
OCH_3-6	3	3.99 (s)
OCH_3-4'	3	3.88 (s)
OAc-5	3	2.52 (s)
OAc-7	3	2.41 (s)

s= singlet, d = doublet ; spectrum run in $CDCl_3$ at 60 MHz, using TMS as internal standard (δ-scale).

Table-11

^{1}H-NMR spectral data of Vc-7Ac

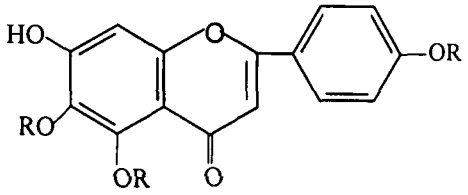
Assignment	No. of Protons	Signets
H-8	1	7.00 (s)
H-3	1	6.62 (s)
H-3',5'	2	7.10 (d, J=9 Hz)
H-2',6'	2	7.75 (d, J=9 Hz)
H-1",2",3",4" (glucosyl) H-1"',2"',3"',4"' (rhamnosyl)	8	4.42-5.50 (m)
H-5", 6" (glucosyl) H-5"' (rhamnosyl)	4	3.40-4.40 (m)
Aliphatic acetoxyl	3	1.90 (s)
5-Aliphatic acetoxyls	15	2.12 (br s)
1-Aromatic acetoxyl	3	2.5 (s)
Aromatic acetoxyl	3	3.96 (s)
1-Aromatic methoxyl	3	3.91 (s)
Rhamnosyl methyl	3	0.88 (d, J=6 Hz)

s=singlet, d= doublet, m = multiplet; br s=broad singlet, spectrum run in $CDCl_3$ at 100 Hz using TMS as internal standard (δ-scale).

Vc-8:

It was crystallized from methanol as yellow needless m.p. >300^{0}C. Elemental analysis agreed with the molecular formula $C_{27}H_{30}O_{16}$. It was proposed to be a flavone glycoside by its positive Shinoda's test, Molish test and **uv** spectrum. The **uv** spectrum showed λ_{max} at 285 and 336 nm and the shifts in the presence of classical shift reagents[18] indicated the presence of free hydroxyls at 5,6,7 and 4'-positions. Acid hydrolysis with 7% HCl yielded an aglycone and glucose. The aglycone on acetylation formed a tetraacetate m.p. 238-39^{0}C and on methylation gave a tetramethylether m.p. 160-62^{0}C. The aglycone was characterized as scutellarein (XI-a) by R_F values, m.p. m.m.p, **uv** spectral behaviour and co-chromatography with an authentic sample. The **uv** spectrum of the glycoside and the aglycone was almost similar except that the aglycone gave a red shift of 15 nm with NaOAc (absent in glycoside) thus suggesting that position 7 is involved in glycosidation.

The position of sugar residue at C-7 in the glycoside was further confirmed by hydrolysis of methylated glycoside. The partial methylether thus obtained was characterized as scutallarcin 5,6,4'-trimethylether (XI-b). It showed a red shift of 11 nm with NaOAc showing the presence of a free hydroxyl ether at C-7 position. The formation of this partial methyether thus proved that glucose is attached to C-7 of the aglycone. The methylated sugars were identified as 2,3,4-tri-O-methyl-D-glucose thus confirming the inter sugar linkage between two glucose units as (1→6) gentiobioside.

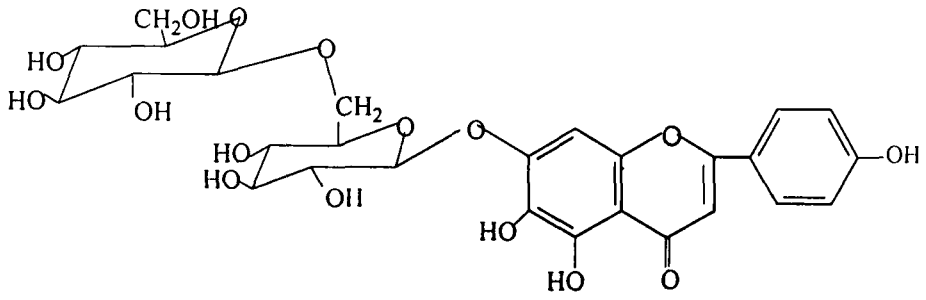


(XI)

(a) : R = H
(b) : R = CH_3

The quantitative estimation of sugar by the method described earlier showed the presence of 2 mole of sugars per mole of the aglycone.

On the basis of above finding, **Vc-8** was characterized as **Scutellarein-7-diglucoside (XII).**



(XII)

EXPERIMENTAL

STUDY OF THE LEAVES OF VIBURNUM CONTINIFOLIUM

The powdered leaves of **Viburnum cotinifolium** (1 Kg) were successively refluxed with petrol and benzene. The defatted leaves were then exhaustively extracted (three times, 3 liters each) by refluxing with methanol. All the methanol extracts were combined together and distilled under reduced pressure. A brownish gummy mass was left behind. TLC examination of the residue in BPF, TEF and EtOAc-EtMeCO-AcOH-H_2O showed the presence of eight major spots along with some minor impurities. The brown gummy mass (20 gm) was subjected to column chromatography over silica gel (2.5 kg) and eluted with benzene-ethylacetate in different proportions (9:1-1:1) and monitored by TLC. The eluates revealed five compounds in varying concentrations. Repeated columns chromatography of the fractions over silica gel column failed to separated the ~~delicate~~ complicated mixture of compounds. Therefore they were separated by preparative TLC using BPF as the solvent system. The fractions thus obtained gave pure **Vc-1** (60 mg), **Vc-2** (75 mg), **Vc-3** (70 mg), **Vc-4** (350 mg), and **Vc-5** (100 mg).

The fractions obtained from EtOAc and EtOAc-MeOH mixture in different ratio (9:1-8:2) revealed two compounds common ~~to them~~, in varying concentrations. The above fractions were therefore mixed together and separated by preparative TLC using EtOAc-EtMeCO-AcOH-H_2O (5:3:1:1) as solvent systems. The homogeneity of the compounds were established and were labeled as **Vc-6** (300 mg) and **Vc-7** (280 mg). Further elution of the column from EtOAc-MeOH (7:3) gave a single compound along with some minor impurities, which were removed by crystallization with benzene-methanol and was labeled as **Vc-8**.

Vc-1:

It was crystallized with $CHCl_3$-MeOH as yellow cubes (60 mg) m.p. 263°C.

Analysed for $C_{15}H_{12}O_6$:

Calcd.: C, 62.50, H, 4.16%

Found : C, 62.66, H, 4.24%

UV with shift reagents, λ_{max} nm:

MeOH	2.86, 326 Sh
$AlCl_3$	309, 336
$AlCl_3$/HCl	304, 375
NaOAc	28. Sh, 320
NaOAc/H_3BO_3	289, 332

^{1}H-NMR (DMSO-d_6) on δ scale:

3.0 (m, 2H, C_3-H) 5.40 (m, 1H, C_2-H), 6.66 (1H,d, J=2.5 Hz H-6), 6.88 (1H,d, J=2.5 Hz, H-8), 7.28 (3H, m, H-2, 2',5',6').

IR, υ^{KBr}_{max} cm^{-1}:

3430, 1680 (>C=O)

Acetylation of Vc-1:

Vc-1 (30 mg) was acetylated by heating it on a water bath with Ac_2O (1 ml) and pyridine (0.5 ml). After usual work up it was crystallized with $CHCl_3$-MeOH as colourless needles m.p. 143-44°C

^{1}H-NMR ($CDCl_3$) on δ-Scale:

6.68 (IH, d, J=2.5 Hz, H-6), 6.90 (1H, d, J=2.5 Hz, H-8), 7.30 (3H, m, H-2',5',6'), 5.40 (1H,q, J_1=12 Hz, J_2=4 Hz, H-2), 3.19 (1H, q, J_1=12 Hz, J_2=17 Hz H-3ax), 2.94 (1H, q, J_1=4 Hz, J_2=17 Hz, H-3eq), 2.35 (H, s, OAc-5), 2.28 (9H, s, OAc-7,3',4').

Vc-2:

Vc-2 was crystallized by ethylacetate-acetone as yellow crystals (75 mg) m.p. > 320°C.

Analysed for $C_{15}H_{10}O_6$:

Calcd.: C, 62.93; H, 3.49%

Found: C, 62.95; H, 3.51%

UV, with shift rreagents, λ_{max} nm:

MeOH	269, 290 sh, 345
NaOMe	296, 329sh, 369
NaOAc	290, 326 sh, 376
$NaOAc/H_3BO_3$	278, 291 sh, 360, 730 sh
$AlCl_3$	276, 304 sh,328, 426
$AlCl_3/HCl$	266, 294 sh, 355, 385

Acetylation of Vc-2:

Vc-2 (40 mg) was acetylated with Ac_2O (1 ml) and pyridine (0.5 ml) by usual method and crystallized from chloroform-methanol as colourles needles m.p. 200°C.

^{1}H-NMR (300 MHz, $CDCl_3$) on δ-Scale:

6.85 (1H, d, J=2.0 Hz, H-6), 7.35 (1H,d, J=2.0 Hz, H-8), 6.61 (1H, s, H-3), 7.76 (1H, dd, J_1=8.0 Hz, J_2=2.20 Hz, H-6'), 7.38 (1H,d, J=8.0 Hz, H-5'), 7.70 (1H, d, J=2.20 Hz, H-2'), 2.43 (3H, s, OAc-5), 2.35 (3H, s, OAc-7), 2.33 (6H, s, OAc-3',4')

IR, υ^{kBr}_{max} cm^{-1}:

3400 (OH), 1640 (>C=O), 800-840 (aromatic nucleus)

Mass, m/z:

454 $[M]^{+\cdot}$, 412 $[M^{+\cdot}-42]$, 370 $[M^{+\cdot}-(2 \times 42)]$, 328 $[M^{+\cdot}-(3 \times 42)]$, 286 $[M^{+\cdot}-(4 \times 42)]$, 153 $[A, +H]^{+\cdot}$, 134 $[B]^{+\cdot}$.

Vc-3:

It was crystallized with benzene-acetone as a yellow needles (70 mg) m.p. 248-50°C.

Analysed for $C_{15}H_{12}O_5$:

Calcd.: C, 66.17; H, 4.41%

found : C, 66.28, H 4.49%

UV, with shift reagents, λ_{max} nm:

MeOH	287, 326 sh
$AlCl_3$	310, 383
$AlCl_3$/HCl	310, 382
NaOAc	283 sh, 382
$NaOAc/H_3BO_3$	289, 330

^{1}H-NMR [300 MHz, $(CD_3)_2CO$] on δ-scale:

6.64 (1H,d, J=2.5 Hz, H-6), 6.86 (1H, d, J=2.5 Hz, H-8), 6.94 (2H, d, J=8.5 Hz, H-3',5'), 7.40 (2H, d, J=8.5 Hz, H-2',6'), 5.20 (1H, q, J_1=12 Hz, J_2=4 Hz, H-2), 2.79-2.98 (2H, m, J_1=12 Hz, J_2=4 Hz, J_3=17 Hz, H-3,3).

Vc-4:

It was crystallized as yellow needles (350 mg) from methanol m.p. 254-55°C.

Methylation of Vc-4:

Vc-4 (100 mg) was methylated by refluxing it with dimethyl sulphate (2 ml) and anhydrous potassium carbonate (2.5 gm) in dry acetone (100 ml).

On usual work up and crystallization from chloroform-methanol, gave white shinning needles m.p. 226-27°C.

^{1}H-NMR (300 MHz, $CDCl_3$) on δ-scale:

6.46 (1H, d, J=2.45 Hz, H-I-8), 6.32 (1H, d, J=2.5 Hz, H-I-6), 6.63 (1H, s, H-II-6), 6.50, 6.57 (1H each, s, H-I-3,H-II-3), 7.94 (1H, q, J_1=9.0 Hz, J_2=2.5 Hz, H-I-6'), 7.85 (1H,d, J=2.5 Hz, H-I-2'), 7.10 (1H,d, J=9.0 Hz, H-I-5'), 7.38 (2H, d, J=9.0 Hz, H-II-2',6'), 6.73 (2H, d, J=9.0 Hz, H-II-3',5'), 3.92, 4.05 (3H each, s, OMe-I-5, H-5), 3.76, 3.72 (3H each, s, Me-I-4', II-4'), 3.90, 3.82 (3H each, s, OMe-I-7, OMe-II-7).

Mass (E1-MS) of Vc-4 Me, m/z:

622 (100), 621 (33), 592 (8), 576 (10), 312 (12), 245 (15), 181 (20), 135 (16).

Acetylation of Vc-4:

Vc-4 (100 mg) was heated with pyridine (1 ml) and Ac_2O (2 ml) on water bath for 2 hours. It was then cooled at room temperature and poured into crushed ice. The separated solid was filtered, washed with water and dried. It was crystallized with $CHCl_3$- MeOH as colourless nedles m.p. 240-42°C.

^{1}H-NMR (100 MHz, $CDCl_3$) on δ-scale:

7.26 (1H,d, J=3 Hz, H-I-8), 6.84 (1H,d, J=3 Hz, H-I-6), 7.01 (1H, s, H-II-6), 7.98 (1H, q, J_1=8 Hz, J_2=3 Hz, H-I-6'), 8.03 (1H, d, J=3 Hz, H-I-2'), 7.46 (1H, d, J=9 Hz, H-I-5'), 7.06 (2H, d, J=9 Hz, H-II-3',5'), 6.68, 6.65 (2H, s, H-I-3, H-II-3), 2.28, 2.23 (3H each, s, OAc-I-4', II-4'), 2.05, 2.01 (3H each, OAc-I-7, II-7), 2.45, 2.41 (3H each, s, OAc-I-5, II-5).

Vc-5:

It was crystallized with chloroform-methanol as pale yellow crystals (100 mg) m.p. 222-23°C.

Analysed for $C_{30}H_{18}O_{11}$:

Calcd: C, 64.19, H, 32.5%

found: C, 64.10, H 32.7%

UV, with shift reagents, λ_{max} nm:

MeOH	270, 290 sh, 336
NaOMe	276, 295, 385
$AlCl_3$	262 sh, 278, 300, 350, 386
$AlCl_3$/HCl	263 sh, 279, 299, 348, 385
NaOAc	274, 293, 369
NaOAc/H_3BO_3	272, 332.

^{1}H-NMR (400 MHz, DMSO-d_6) on δ-scale:

6.19 (1H, s, H-I-3), 6.38 (1H,s, H-II-3), 6.78 (1H, s, H-II-6), 6.80 (1H, s, H-I-8), 6.63 (2H, d, J=8.5 Hz and 2.0 Hz, H-I,3',5'), 7.60 (2H, d, J=8.5 Hz and 2.0 Hz, H-I-2',6'), 7.02 (2H, d, J=8.5 Hz and 2.0 Hz, H-II-3',5'), 7.90 (2H, d, J=8.5 Hz, and 2.0 Hz, H-II-2',6').

Mass, m/z:

$M^{+\cdot}$ 554 (5%), 121 (6.8%), 118 (12.8%), 318, 290 (8%), 300 (4.8%), 193 (8.8%), 285 (17.9%), 436, 270, 178 (6.8%), 152 (4..5%), 334 (2.0%), 326 (7.6%), 299 (8.4), 418 (8.7%), 390 (8.4%).

Acetylation of Vc-5:

Compound **(Vc-5)** (25 mg) was acetylated by heating it over a water bath with pyridine (1 ml) and acetic anhydride (2 ml) for 3 hours. After usual work up it was crystallized with chloroform-ethanol as white crystals (15 mg) m.p. 155°C.

^{1}H-NMR ($CDCl_3$) on δ-scale:

2.45(6H, s, OAc-I-5, II-5), 2.35 (6H, s, OAc-I-5, II-7), 2.33 (6H, s, OAc-I-4, II-4), 6.23 (1H, s, H-I-3), 6.45 (1H, s, H-II-3), 6.79 (1H, s, H-II-6), 6.85 (1H, s, H-I-8), 6.70 (2H, d, J=8.5 and 2.0 Hz, H-I-3′,5′), 7.69 (2H, d, J= 8.5 and 2.0 Hz, H-I-2′,6′), 7.08 (2H, d, J=8.5 and 2.0 Hz, H-II-3′,5′), 7.95 (2H, d, J=8.5 and 2.0 Hz, H-II-2′,6′).

Methylation of Vc-5:

A mixture of **Vc-5** (35 mg), anhydrous potassium carbonate (3 gm), dimethyl sulphate (1 ml) and dry acetone (100 ml) was refluxed on water bath for about 12 hours. A small portion of the reaction mixture was taken out in a test tube and tested with alcoholic $FeCl_3$. Refluxing continued until it gave a negative alcoholic $FeCl_3$ test. It was then filtered and the residue washed several time with hot acetone. The filtrate and the washings were combined and evaporated to dryness. The yellow residue was washed 2-3 times with petroleum ether and then taken up in chloroform (100 ml) into a separating funnel and washed several times with water. The chloroform solution was dried over anhydrous sodium sulphate, concentrated and purified on a silica gel column using chloroform as the eluant. It was finally purified by preparative TLC to yield a white solid which crystallized from chloroform-methanol as colourless needles (20 mg) m.p. 150°C.

^{1}H-NMR (300 MHz, $CDCl_3$) on δ-scale:

4.00 (3H, s, OMe-II-5), 3.88 (3H, s, OMe-I-5), 3.86 (3H, s, OMe-I-7), 3.76 (3H, s, OMe-II-7), 3.74 (3H, s, OMe-I-4′), 3.59 (3H, s, OMe-II-4′), 6.21 (1H, s, H-I-3), 6.40 (1H, s, H-II-3), 6.77 (1H, s, H-II-6), 6.82 (1H, s, H-I-8), 6.66 (2H, d, J=8.5 Hz & 2.0 Hz, H-I-3′,5′), 7.61 (2H, d, J=8.5 Hz & 2.0 Hz, H-I-2′,6′), 7.06 (2H, d, J=8.5 Hz & 2.0 Hz, H-II-3′,5′), 7.92 (2H, d, J=8.5 Hz & 2.0 Hz, H-II-2′,6′).

Vc-6:

The compound **(Vc-6)** was crystallized from ethylacetate-alcohol as pale yellow needles (300 ml), m.p. >275°C.

Analysed for $C_{28}H_{32}O_{15}$:

Cacd.: C, 55.26; H, 5.26%

found: C, 55.38; H, 5.23%

UV with shift reagents, λ_{max} nm:

MeOH	290, 337
+$AlCl_3$	270 sh, 316, 364
+$AlCl_3$/HCl	269 sh, 314, 360
+NaOMe	288, 300 sh, 360
+NaOAc	299, 375
+NaOAc/H_3BO_3	290, 330 sh, 340

IR, υ^{KBr}_{max} cm^{-1}:

3450 (OH), 1650 (C=O)

Acetylation of Vc-6:

Vc-6 (40 mg) was dissolved in pyridine (0.5 ml), and acetic anhydride (1 ml) and left overnight, before work out it was heated over a boiling water bath for 15 minutes. The reaction mixture was cooled and crushed ice was added to it. The solid thus separated was filtered, washed well with water and crystallized with alcohol, m.p. 166-68°C

^{1}H –NMR ($CDCl_3$) on δ-scale:

8.07 (2H, d, J=9 Hz, H-2',6'), 7.40 (2H, d, J=9 Hz, H-3',5'), 6.87 (1H, s, H-8), 6.65 (1H, s, H-3), 3.79 (3H, s, OCH_3), 2.35, 2.48 (3H each s, OAc-7,5), 2.02- 2.09 (18H, m, 6 x OAc), 3.40-5.44 (sugars), 0.88 (3H, d, J=6 Hz, rhamnosyl-CH_3).

Acid hydrolysis of Vc-6:

Vc-6 (100 mg) was dissolved in water (100 ml) and HCl (7 ml) was added to it. The mixture was heated for 2 hours on a boiling water bath. The yellow solid thus separated was filtered, washed well with water and dried. It was crystallized from methanol as yellow needles, m.p. 268-70^{0}C.

Analysed for $C_{16}H_{12}O_6$:

Calcd: C, 64.00; H, 4.00%

Found: C, 64.13; H, 3.95%

UU with shift reagents, λ_{max} nm:

MeOH	284, 334
+$AlCl_3$	301, 384
+NaOMe	303, 386
+NaOAc	299, 365

Acetylation of the aglycone:

The aglycone (30 mg), was dissolved in pyridine (0.5 ml) and acetic anhydride (1 ml) was added to it. The mixture was heated on a water bath for 2 hours. On usual work up, it gave dirty, white precipitate, which on crystallization with ethanol gave white needles m.p. 227^{0}C.

Analysed for $C_{22}H_{18}O_9$:

Calcd: C, 61.97; H, 4.22%

Found: C, 61.45; H, 4.00%

Methylation of the aglycone:

The aglycone (35 mg) was refluxed in dry acetone (50 ml) with dimethyl sulphate (0.8 ml) and anhydrous potassium carbonate (1 gm) for 48 hours. After usual work up a cream coloured solid (20 mg) was obtained on

several crystallizations from chloroform-methanol it gave colourless needles m.p. 160-61^{0}C.

Analysed for $C_{19}H_{18}O_6$:

Calcd: C, 66.66; H, 5.26%

Found: C, 66.49; H, 5.18%

Identification of sugars:

The aqueous hydrolysate was concentrated to a syrup in vacuum over KOH pellets. The sugars were identified by paper chromatography in two different solvent systems namely, n-butanol-acetic acid-water (4:1:5, upper layer) and n-butanol-water-ethanol (60: 28.5:16.5) using an authentic sugars as check. The R_f-values of the sugars were identical with those of glucose (0.18, 0.10) and rhamnose (0.37, 0.28).

6-Hydroxy-4', 5,7-trimethoxy flavone:

CH_3I (1 ml) and Ag_2O (300 mg) were added to the solution of the glycoside (30 mg) in DMF (3 ml). The mixture was stirred in dark at room temperature for 48 hours. The contents were filtered and the residue was washed with little of DMF. The filtrate was evaporated to dryness and the residue was treated with ethanol (25 ml). The alcohol was filtered and evaporated, syrupy residue was left behind. The residue was hydrolysed by heating with 0.3 N HCl. On usual work up it gave light yellow solid, crystallized by ethylacetate m.p. 220-21^{0}C.

Analysed for $C_{18}H_{16}O_6$:

Calcd: C, 65.85; H, 4.87%

found: C, 65.78; H, 4.82%

Estimation of Sugar:

The glycoside (40 mg) was hydrolysed by refluxing it for two hours with 2% H_2SO_4. After cooling overnight, the aglycone was filtered, washed dried and weighed (17.8 ml). The ratio of the aglycone to the glycoside is

44.7% and this ratio indicates the presence of two moles of sugar per mole of the aglycone.

Somogyi's copper micro method gave the value (1.67cc) which corresponded to two moles of sugars per mole of the aglycone.

Vc-7:

It was crystallized from ethylacetate-methanol mixture as yellow needles (280 mg), m.p. 248-250^{0}C.

Analysed for $C_{29}H_{34}O_{15}$:

Calcd: C, 56.03; H, 5.31%

Found: C, 56.18; H, 5.29%

UV with shift reagents, λ_{max} nm:

MeOH	267, 335
+$AlCl_3$/HCl	272 sh, 289, 350, 406 sh
+NaOAc	274, 378
+NaOMe	275, 292 sh, 380

IR, υ^{KBr}_{max} cm^{-1}:

3400, 1650, 840

Acetylation of Vc-7:

The glycoside (50 mg) was heated with acetic anhydride (1 ml) and pyridine (0.7 ml) over a water bath for 3 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid separated was filtered, washed with water and dried. On several, crystallization from ethanol it gave shining plates, m.p. 136-38^{0}C.

^{1}H-NMR (100 MHz, $CDCl_3$) on δ-scale:

7.00 (1H, s, H-8), 7.10 (2H, d, J=9 Hz, H-3',5'), 7.75 (2H, d, J=9 Hz, H-2',6'), 6.62 (1H, s, H-3), 4.54 (1H, d, J=2.5 Hz, H-1'''), 5.30 (1H, d, J=8 Hz,

H-1"), 3.40-5.50 (12H, m, rhamnoglucosyl protons), 1.90 (3H, s, OAc), 2.12 (15H, broad s, 5 x OAc), 3.96 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 0.88 (3H, d, J=6 Hz, rhamnosyl-CH_3), 2.50 (3H, s, OAc).

Hydrolysis of the glycoside:

The glycoside (150 mg) was dissolved in water (50 ml) and heated with HCl (4 ml) over a boiling water bath for 2 hours. The mixture was left overnight, the aglycone thus separated out was filtered, washed with water and dried. It was crystallized with methanol as pale yellow needles (120 mg), m.p. 214-16^{0}C.

Analysed for $C_{17}H_{14}O_6$:

Calcd: C, 64.96; H, 4.45%

Found: C, 64.89; H, 4.38%

UV with shift reagents, λ_{max} nm:

MeOH	275, 331
+$AlCl_3$/HCl	270 sh, 290, 350 405 sh
+NaOAc	293, 382
+NaOMe	277, 294 sh, 380

Acetylation of the aglycone:

The aglycone (30 mg) pyridine (0.5 ml) and acetic anhydride (1 ml) were heated on water bath for 2 hours. After usual work up, the product was crystallized from methanol as colourless needles, m.p. 110-12^{0}C.

Analysed for $C_{14}H_{18}O_8$:

Calcd: C, 63.32; H, 4.52%

Found: C, 63.19; H, 4.49%

^{1}H-NMR (60 MHz, $CDCl_3$) on δ-scale:

7.01 (1H, s, H-8), 6.50 (1H, s, H-3), 6.85 (2H, d, J=9 Hz, H-3',5'), 7.60 (2H, d, J=9 Hz, H-2',6'), 3.88 (3H, s, OCH_3-4'), 3.99 (3H, s, OCH_3-6), 2.41 (3H, s, OAc-7), 2.52 (3H, s, OAc-5).

Methylation of the aglycone:

The aglycone (50 mg) was dissolved in dry acetone (50 ml). Dry and pure dimethyl sulphate (0.3 ml) and fused potassium carbonate (2 gm) were added to the solution. The reaction mixture was refluxed for 72 hours, till it gave negative test with ferric chloride. After usual work up, the solid obtained was crystallized from methanol as cream coloured needles (35 mg) m.p. 161-63^{0}C.

^{1}H-NMR ($CDcl_3$) on δ-scale:

6.70 (1H, s, H-8), 6.90 (2H, d, J=9 Hz, H-3',5'), 7.70 (2H, d, J=9 Hz, H-2',6'), 3.80 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.90 (6H, s, 2 x OCH_3), 6.45 (1H, s, H-3).

Identification of sugars:

The neutral aqueous hydrolysate on paper chromatographic examination with authentic sugars showed the presence of glucose & rhamnose as the sugar moieties.

Vc-8:

It was crystallized with methanol as light yellow needles (700 mg), m.p. > 300^{0}C.

Analysed for $C_{27}H_{30}O_{16}$:

Calcd: C, 53.11; H, 4.91%

Found: C, 53.21; H, 4.99%

UV with shift reagents, λ_{max}nm:

MeOH	285, 336
+$AlCl_3$/HCl	272 sh, 294, 363

+NaOAc	271, 295, 361
+NaOMe	280, 363
+NaOAC/H_3BO_3	286, 340
+NaOMe	288, 380

Acid hydrolysis of Vc-8:

Vc-8 (100 ml) on heating with 7% HCl over a water bath for two hours gave scutelliarien, it was crystallized with ethanol as pale yellow needles (60 mg), m.p. >340^{0}C.

Analysed for $C_{15}H_{10}O_6$:

Calcd: C, 62.93; H, 3.49%

Found: C, 62.84; H, 3.44%

Acetylation of Scutellarien:

Scutellarien (20 mg) was acetylated with Ac_2O and pyridine by heating it at 100^{0}C for 2 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid was washed well with water and dried. On crystallization with ethanol it gave cream coloured needles (25 mg) m.p. 238-39^{0}C.

Analysed for $C_{17}H_{14}O_{10}$:

Calcd: C, 60.79; H, 3.96%

Found: C, 60.66; H, 3.84%

Methylation of scutellarien:

Scutellarien (50 mg) was refluxed in dry acetone (20 ml) with dimethyl sulphate (1.0 ml) and anhydrous potassium carbonate (2 gm) for 48 hours. After usual work up the solid obtained was crystallized. From $CHCl_3$-MeOH as colourless needles (25 mg) m.p. 160-62^{0}C.

Analysed for $C_{19}H_{18}O_6$:

Calcd: C, 66.66; H, 5.26%

Found: C, 66.78; H, 5.31%

Permethylation of Vc-8: (Hakomoris method)

NaH (250 mg) was stirred with DMSO (15 ml) at 80^0C for 30 minutes under N_2 gas. To this reagent, solution of the glycoside (50 mg) in DMSO (1 ml) was added. The stirring was continued for half an hour more. The reaction mixture was poured into ice-water and extracted with EtoAc, washed with water, and dried. The product obtained was purified by PTLC using C_6H_6-Me_2Co (4:1) as the developing solvent. The permethylated product obtained was crystallized from $CHCl_3$-MeOH as colourless needles. (32 mg).

7-hydroxy-4',5,6-trimethoxy flavone:

The permethylated glycoside on hydrolysis with killianis mixture (HOAC-HCl-H_2O 7:3:10) gave 7-hydroxy-4',5,6-trimethoxy flavone (scutellarein trimethyl ether) m.p. 180-84^0C, 2,3,4-tri-O-methyl-D-glucose and 2,3,4, 6-tetra-O-methyl-D-glucose (TLC, Silica gel, toluene-methanol , 4:1).

Analysed for $C_{18}H_{16}O_6$:

Calcd: C, 65.85; H, 4.87%

Found: C, 65.96; H, 4.91%

UV with shift reagents, λ_{max}nm:

MeOH	279, 340
+NaOAc	290, 365

Identification of sugar by paper chromatography:

The aqueous hydrolysate was neutralized as described earlier. It was examined by paper chromatography employing n-butanol-HOAC-H_2O (4:1:5) as developing solvent and the authentic sugars as check. The chromatograms

were developed and sprayed with aniline hydrogen pthalate. The chromatograms on drying at 100-105^0 showed the presence of only glucose.

Estimation of sugar:

The anhydrous glycoside (22 mg) was hydrolysed by refluxing it for 2 hours with 2% H_2SO_4. After cooling overnight, the aglycone was filtered and dried (10 mg). The ratio of the aglycone to the glycoside is 45.4% indicating the presence of 2 moles of sugar / mol of aglycone.

REFERENCE

1a. '**The wealth of India**,' Raw Material, CSIR, New Delhi: Vol X, p. 457 (1976).

b. S. Chavollean, A. Debal and E. Uciani, **Rev. Fr. Crops Graz 39 (1-2)**. 3-81(Fr) 1992).

2. Testsno, Y. Signetoshi, M. Tsunao, O. Tsutomu, K. Jujo, **Phytochemistry, 31 (4)**, 1311-15 (1992).

3. A.K. Nadhkarni, '**Indian Materia Medica'** Vol.1, 1271 (1976).

4. P.R. Gilreath, J.P. Gileath, **J. Environ, Mortic 4(4)**, 52-6 (1986).

5. A. Lostein, G. Haan-Arichipoff, Englert, T-G. Ku-hry, RM Anton, **Phytochemistry, 50**, 1175-80 (1999).

6. J.B. Harborne, T.J. Mabry and H. Mabry., **The Flavonoids** (Champmon and Hall, London) 46 (1970).

7. P.L. Majumdar & A. Bagchi, **J. Ind. Chem. Soc., 58 (11)**, 1121-2 (Eng) (1981).

8. S.K. Srivastava, S.D. Srivastava and K.P. Tiwari. **Ind. J. Chem. Sect B. 20 (4)**, 347-8 (Eng.) (1981).

9. J. Shinoda, **J. Chem. Pharm. Soc. Japan, 48**, 214 (1925).

10. K. Weiges and R. Kolb, **Phyttochemistry, 10**, 829 (1971).

11. J.B. Harborme, '**Comparative Biochemistry of the Flavonoids**', Academic Press London (1967).

12a S.S. Subramanian, N.G.R. Nair, **J. Ind. Chem. Soc, 49**, 825 (1982).

12b. P. Kloss, **Naturwissenchaften, 54**, 472 (1967).

13. A. Pelter, R. Warren, J.W. Osmani, R.H. Rizvi, M. Ilyas and W. Rehman **Experentia 25**, 350 (1969).

14. S. Natarajan, V.V.S. Murti and T.R. Seshadri, **J. Ind. Chem. Soc., 7,** 751 (1969).

15. L. Horhammer, H. Wagner, H and H. Reinherdt, **Naturwissenchaften 52,** 161 (1965).

16. A. Pelter, R. Warren, N. Hameed, N.U. Khan, M. Ilyas and W. Rahman **Phytomistry 9,** 1897 (1970).

17. T.A. Geissmann, **'The Chemistry of Flavonoids Compound'**, P.No. 72, Pergmon Press, New York, Paris (1962).

18. T.J. Mabry, K.R. Markhan and M.B. Thomas. **'The systematic identification of flavonoids'** springer verlag, New York, Heidelburg (1970).

19a. A.K. Varshaey, **Ph.d. Thesis**, Department of Chemistry, A.M.U., Aligarh. 19, April (1974). W. Rahman ?

19b. N.U. Khan, W.H. Ansari, J.N. Usamni, M. Ilyas and W.R. Rahmani, **Phytochemistry, 10**, 2129 – 2131 (1971)

20. J. B. Harborme and C.A. Williams **in the flavonoids**.

21. T.N.C. Vendantham, S.S. Subramanyam and J.B. Harborne, **Phytochemistry, 16**, 294 (1979).

22. M. Somogyi, **J. Biol. Chem.**, **195** 19 (1952).

23. H. Rosler, T. J. Mabry, M. F. Crammer and J. Kagah, **J. Org. Chem., 30** 4346 (1965).

24. F. Petak, **Bull. Soc.** Fr. 263-6 (1965).

25. K.R. Markhan, **'Techniques of flavonoid identification'**, pp. 36-51, Academic Press, London, (1982).

26. H. Rosler, T. J. Mabry, M. F. Craumer and J. Kagan, **J. Org. Chem., 30,** 4346 (1965).

CHAPTER-V

CARYOTA URENS

Results &

DISCUSSION

CHEMICAL CONSTITUENTS FROM THE BASE LEAVES OF CARYOTA URENS (PALMAE)

The genus **Caryota** comprises 15 species distributed in the tropical parts of India, Burma, Ceylon, Malaysia and Northern Australia. Out of these, three species are reported in India[1] of which **C. urens** is of economic importance.

Caryota species have been reported for their medicinal properties such as internally nutritious and aphrodisiac and also laxative.[2] Earlier investigations on this plant reported the isolation of Amino acids, sugars, ascorbic acid[3], fatty acids, Kernel lipids[4] and sugarsin.[5]

Medicinal importance and scanty work on this plant accelerated our interest to carryout the comprehensive study of the plant **Caryota urens**. The present discussion deals with the isolation and characterization of following compounds. From base leaves of **Caryota urens**.

1. **Triacontane**
2. **Lupeol**
3. **Myricadiol**
4. **β-sitosterol.**
5. **Tetracosonid**
6. **Ursolic acid**
7. **Sorbifolin 6-O-glucoside**
8. **5,7-dihydroxy, 4'-O-methylflavone**

The defatted base leaves of **Caryota urens** (2 Kg) procured from fort of A.M.U., Aligarh, India were extracted exhaustively with petroleum ether ($60\text{-}80^0$) and benzene. The base leaves were then extracted with methanol (3 liters x 3) at room temperature and finally on a steam bath.

The petroleum ether and benzene extracts of the base leaves were divided into neutral (I) and the acidic (ii) parts by treatment with alkali. Chromatographic resolution of the neutral part gave four products **C_Y-1, C_Y-2, C_Y-3, C_Y-4**. The chromatographic resolution of alkali soluble part (ii) over alummina column gave two compounds **C_Y-5** and **C_Y-6**.

Both the hot and cold extracts of methanol showed almost same spots on TLC examination in different solvent systems and therefore were mixed together and concentrated under reduced pressure. The resultant mass was refluxed with petrol, benzene, chloroform, ethylacetate respectively and finally with acetone.

The ethylacetate and acetone concentrates on TLC examination in different solvent systems viz. TEF (5:4:1), BPF (36:9:5) and EtOAc: EtMeCO: AcOH: H_2O (2:3:1:1, 5:3:1) showed two compounds having same R_f values with varying concentrations. They were therefore, combined and subjected to column chromatography over silica gel column using benzene-ethyacetate mixture as eluting solvent in different ratios (9:1 to 1:1). The two compounds thus separated were purified by repeated column chromatography and marked as **C_Y-7** and **C_Y-8**.

C_Y-1:

Hexane yielded **C_Y-1** m.p. 62-67^{0}C. It was found to be identical with **triacontane** on the basis of its elemental analysis ($C_{30}H_{62}$) and **infrared** spectrum, υ^{kBr}_{max} 2930 and 2860 cm^{-1} (C-H, saturated), 1460 and 1380 cm^{-1} (C-CH_3) and 720 cm^{-1} $(CH_2)_n$. Finally, it was analysed by **gas ~~liquid~~ chromatography** which indicated **C_Y-1** to be a mixture of n-alkanes of series C_{24}-C_{36}, **(Fig-I)** containing mainly n-tri-triacontane (28.9%), n-nonacosane (21.2%), n-triacontane (2.9%) accompanied by hexacosane, pentacosane and pentatriacontane as minor consitituents[6].

C_Y-2:

Petroleum ether-benzene (1:4) solution from the neutral part afforded **C_Y-2**, melting point 214-15^{0}C, $[\alpha]^{20}_D$ + 23.64 ($CHCl_3$). It gave positive Liebermann-Burchard and Noller's[7] tests and yellow colour with tetranitromethane. Elemental analysis agreed with the formula $C_{30}H_{50}O$. **Infrared** showed bands at 3360 and 1030 cm^{-1} (OH), 1645 cm^{-1} (C=C) and 1385 cm^{-1} (geminal dimethyl), 885 cm^{-1} (terminal methylene) **(Fig-II)**. **Mass** spectrum of the triterpene alcohol gave $M^{+\bullet}$ at m/z 426 (11%) with other principal ions m/z 411 / (M-CH_3) (6%), 207 (34%), 189 (77%), and a base peak at m/z 95. It afforded an acetate m.p. 218-220^{0}C. **Infrared** spectrum of the acetate revealed the presence of terminal methylene, by a band at 875 cm^{-1}. Some other important bands were observed at 1245 cm^{-1} (acetate), 1640 cm^{-1} (C=C) and 1730 cm^{-1} (C=O). **^{1}H-NMR** spectrum **(Fig-III)** gave the signals at δ 0.82, 0.87, 0.94, 1.04 (CH_3 protons), 1.27, 1.41, 1.46, 1.470 (CH_2 protons), δ 2.03 ($OCOCH_3$) and multiplets at δ 4.28 and 4.77 (>C CHOAc), δ 4.59, 4.67 (>C=CH_2). On the basis of the above physico-chemical data of the compound and its derivatives the **C_Y-2** was identified as **lupeol[8] (I)**.

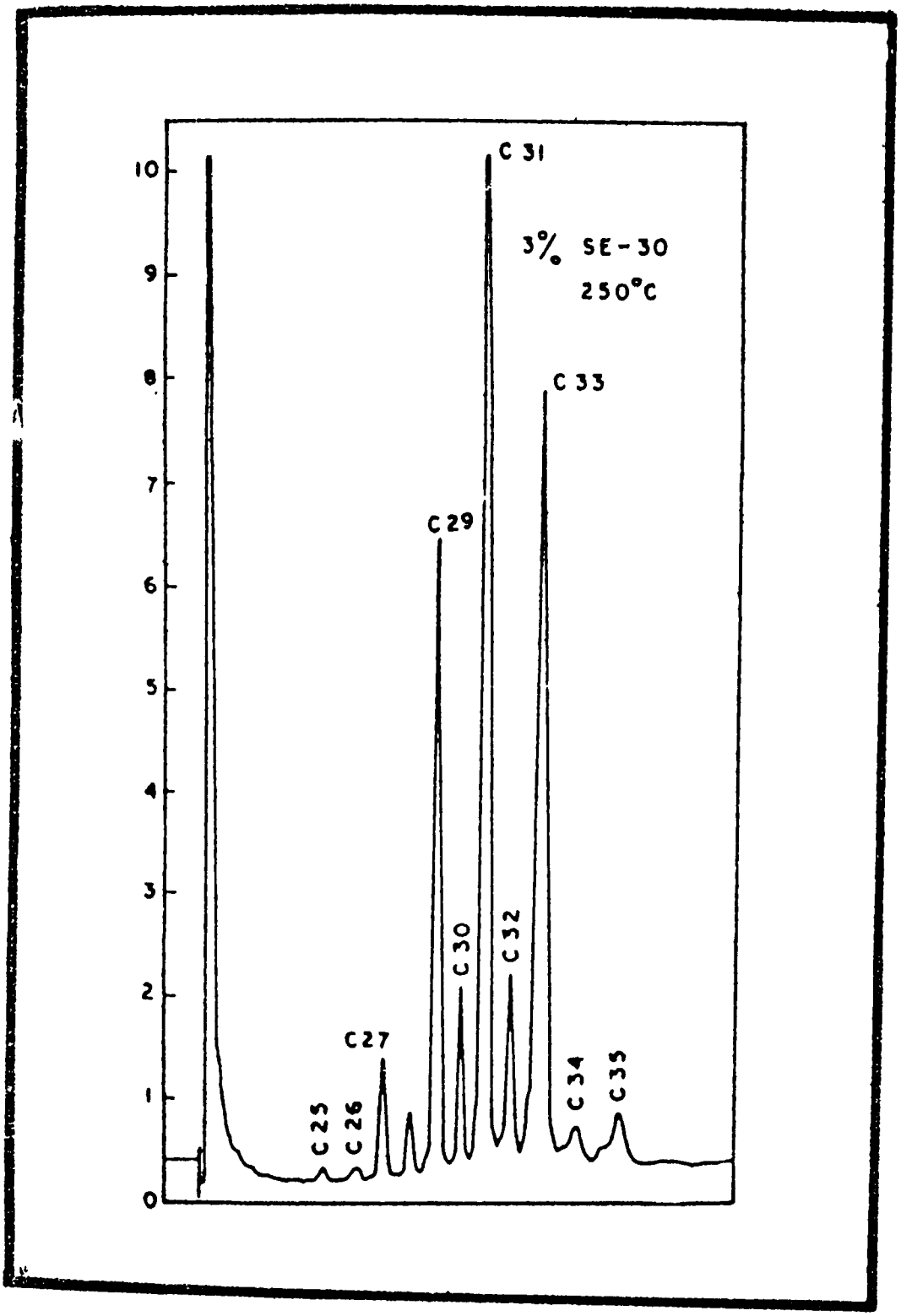
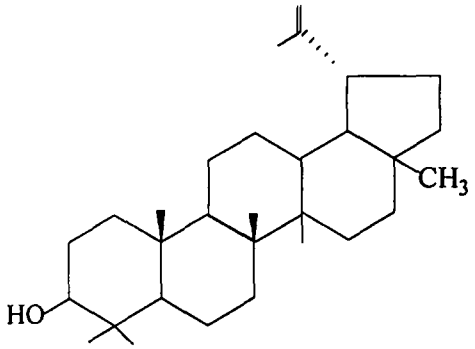

C 31
3% SE-30
250°C
C 33
C 29
C 30
C 32
C 27
C 25
C 26
C 34
C 35
10
9
8
7
6
5
4
3
2
1
0

Fig.-I

Fig.-11

Fig.-III



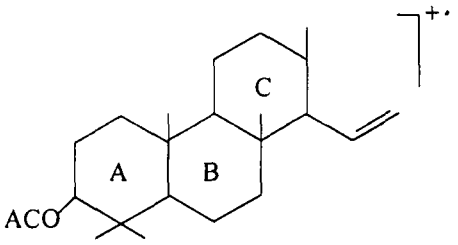
(I)

C_Y-3:

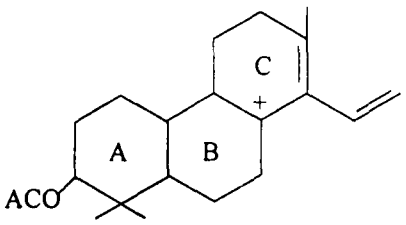
Elution of the column with benzene followed by crystallization from benzene-ethylacetate gave a colourless amorphous compound (**C_Y-3**), m.p. 259-60^{0}C. It gave positive Liebermann-Burchard test showing it to be a triterpene which was confirmed by stannic chloride test. Its **ir** Spectrum (KBr) showed the bands at 3415 (OH), 2940 (unsaturation), 2880, 1470, 1450, 1390, 1380 (characteristic of triterpenic skeleton), 1080, 1030 (C-O stretching and O-H in plane deformation of secondary alcohol) and 815 cm^{-1} **(Fig-IV).**

C_Y-3 on **mass** spectrometric analysis **(Fig-V)** showed a molecular ion peak at m/z 442 revealing its molecular formula to be $C_{30}H_{50}O_2$, further substantiated by elemental analysis. The other abundant ion peaks observed were 442 {$M^{+\cdot}$-18 (H_2O)}, 409 ($M^{+\cdot}$-H_2O-CH_3), 339, 302, 287, 271, 245 (100), 220, 203, 202, 189.

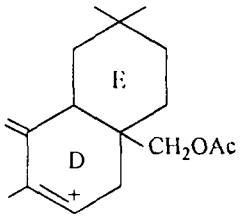
The peaks at m/z 302 and 189 distinguished it to be having a Δ^{14} characteristic i.e. taraxerene skeleton. **C_Y-3** on acetylation formed a diacetate, m.p. 245-47^{0}C. Its **mass** spectrum **(Fig-VI)** showed the molecular ion peak at m/z 526 ($C_{34}H_{54}O_2$) confirming it to be a diacetate and hence in turn **C_Y-3** to be a diol with one primary alcoholic group and one secondary alcoholic group **(ir)**, other ion peaks observed at m/z 511 ($M^{+\cdot}$-CH_3), 466 ($M^{+\cdot}$-HOAc), 334(a), 329(b), 284 (a-HOAc), 269 (b-HOAc), 262 (c), 202 (c-HOAc) and 189 (100) (d); tallied with those of myricadiol diacetate[9], can be explained as shown in the **(scheme-I)**



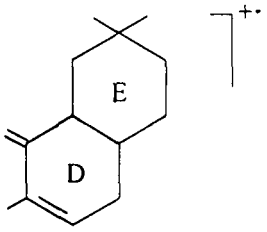
(a)



(b)

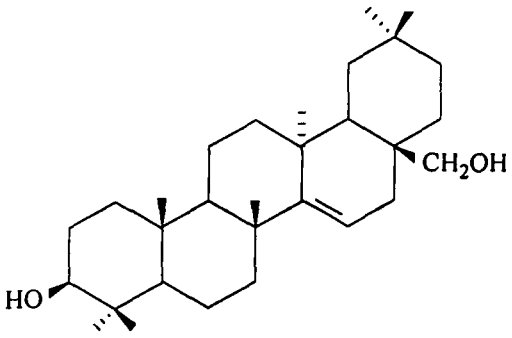


(c)



(d)

On the basis of m.p., acetate, **ir** and **EIMS** studies of C_Y-**3** and its diacetate, the C_Y-**3** was identified as **Myricadiol (II).**



(II)

Fig.-IV

INTENSITY
0
1000
250
300
350
400
450 M/E
414
0.00
4.50
%
INTENSITY
0
1000
50
100
150
200
M/E
0.00
4.50
%

Fig.-V

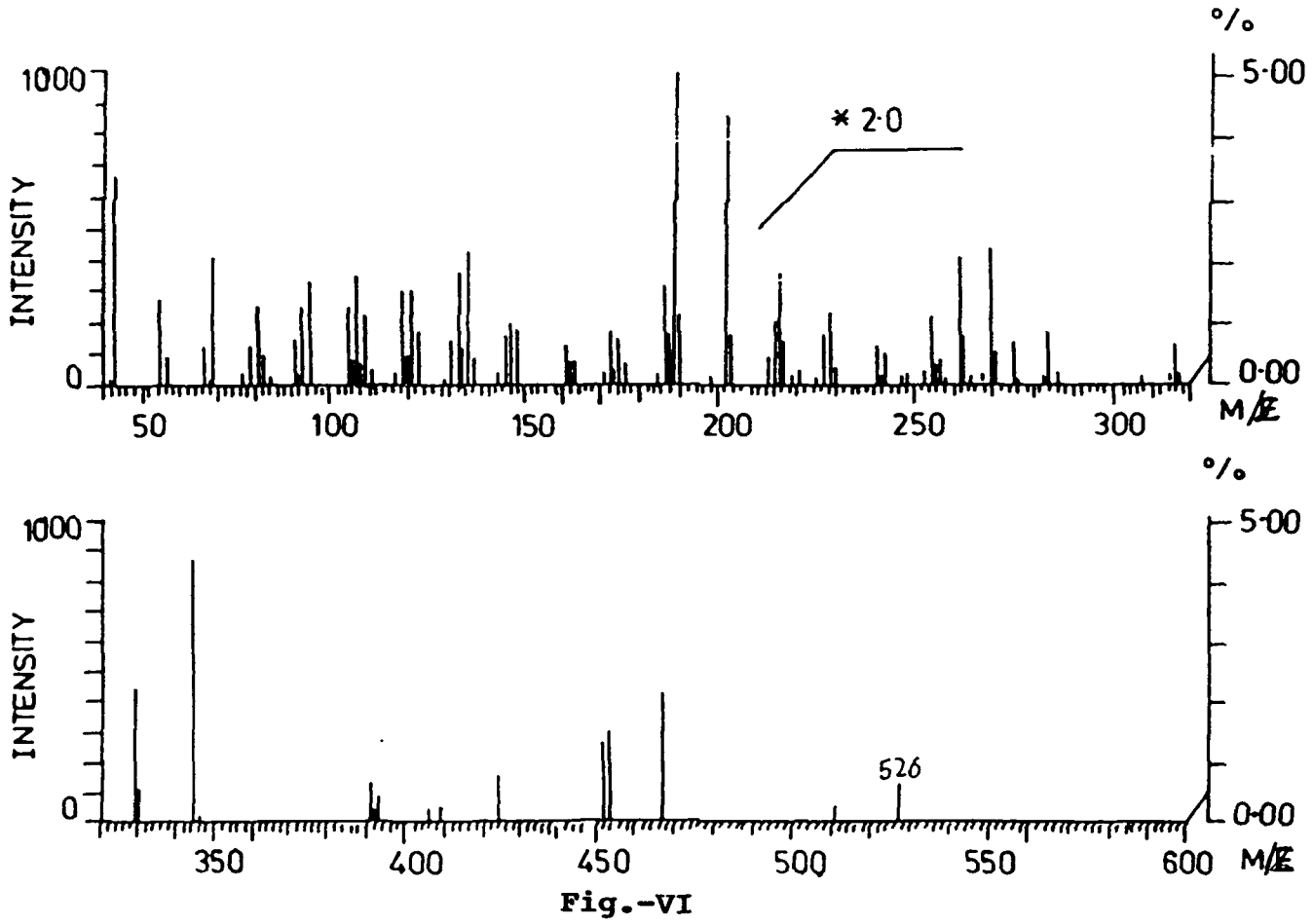
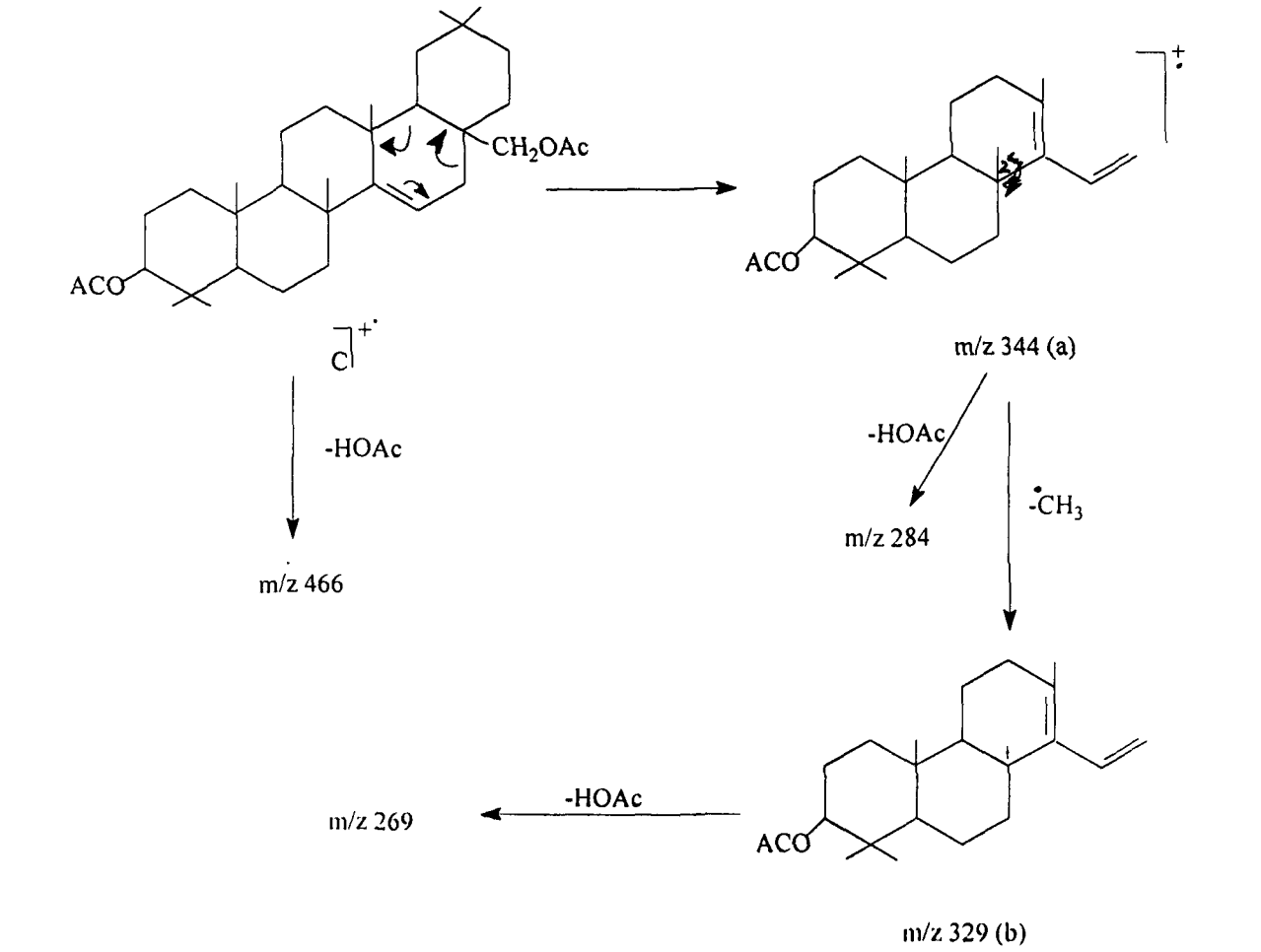
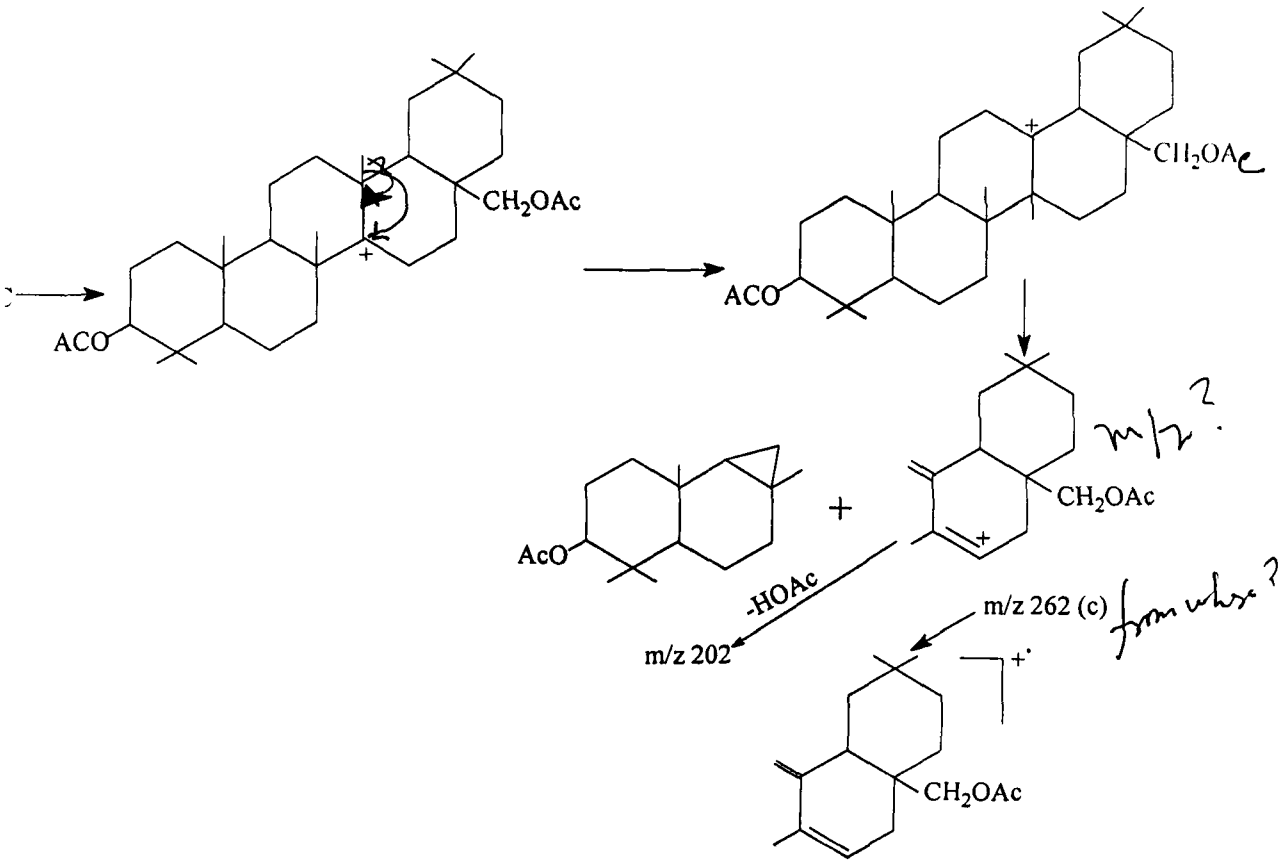

%
1000
5·00
INTENSITY
* 2·0
0
0·00
50
100
150
200
250
300
M/E
%
1000
5·00
INTENSITY
526
0
0·00
350
400
450
500
550
600
M/E

Fig.-VI


CH2OAc
ACO
m/z 344 (a)
-HOAc
m/z 466
-HOAc
m/z 284
·CH3
-HOAc
m/z 269
m/z 329 (b)


CH2OAc
ACO
AcO
+
-HOAc
m/z 202
m/z 262 (c)
CH2OAc
m/z 189 (d)

(Scheme-I)

C_Y-4:

Benzene and chloroform (1:1) eluate afforded another white crystalline **C_Y-4** compound m.p. 159^{0}C, $[\alpha]^{20}_D$ – 53.48^0 ($CHCl_3$). It gave positive Liebermann-Burchard test and responded to tetranitromethane colour test. **IR** spectrum **(Fig-VII)** showed the band at 3350 and 1050 cm^{-1} (OH) and at 1655 and 840 cm^{-1}(C=C). The **^{1}H-nmr** spectrum **(Fig-VIII)** indicated signals at δ 0.7, 0.80, 0.88, 1.02 (CH_3 protons), 3.56 (3α-hydroxyl) and at δ 5.36 (1H, vinyl proton). Spectral data and elemental analysis ($C_{29}H_{48}O$) suggested it to be a **β-sitosterol**. Its acetate m.p. 126^{0}C gave **ir** bands at 2990, 2880, 1740, 1680, 1470, 1390, 1262 and 970 cm^{-1}. Further derivatisation led to the preparation of benzoate, m.p. 144-45^{0}C and 3,5-dintro benzoate m.p. 208-12^{0}C.

For final confirmation **gc-ms (Table-1)** analysis were performed, using a 2.54 x 4 m. I.D. Glass column of 1% Dexil 300 G.C. on 100-120 Diatomic CQ at 260, flow rate 40 ml/min (helium carrier gas) connected through a silicone rubber membrane into an AEIMS-9 **mass** spectrometer **(Fig-IX)**. This has been found to consist of the following four components.

Table -1

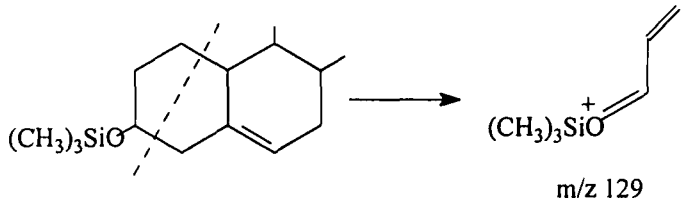
GLC data of sterols (TMS derivatives) ~~on Dexsil 300 G.C.~~

Components	% sterol	RRT**
Cholesterol	0.4	0.60
Campesterol*	11.0	0.81
Stigmasterol*	41.5	0.85
β-sitosterol	47.0	1.0

*Neither **glc** nor **ms** techniques are able to distinguish between sterol C_{24} epimers and these compounds may be either named compounds or its C_{24} epimers.

** Relative retention time (RRT) is expressed by the ratio of retention time for the substance under examination to the retention time of β-sitosterol.

The TMS ether of cholesterol, campesterol, stigmasterol and β-sitosterol gave molecular ions at m/z 458 (24%), 472 (25%), 484 (59%) and at m/z 486 (30%) respectively **(Table-2)**. The characteristic peak at m/z 129 at Δ^5 3β-trimethyl silyloxy steroid for all sterols. The peak at 129 has been identified as the fragment originating from the cleavage of ring-A along with the TMS moiety.



m/z 129

Similarly, the other characteristic fragmentation from Δ^5 3β-trimethyl silyloxy steroid, as reported by Brook,[10] was series of ions from $M^{+\cdot}$-129. These ions were also prominent at m/z 329 (100), 343 (100), 355 (36) and 357 (100) in the mass spectra of cholesterol, campesterol, stigmasterol and β-sitosterol trimethyl-silyloxy derivatives respectively.

The structural features which distinguish each of these sterols is the side chain, of cholesterol contain C_8H_{17} chain, campesterol has a C_9H_{19} chain, stigmasterol has $C_{10}H_{19}$ chain, due to the presence of double bond at carbon 22, β-sitosterol has a $C_{10}H_{20}$ chain. The peaks at m/z 255, 275 were due to the loss of TMS and side chain moieties from the parent compounds of cholesterol and campesterol respectively.

Table-2

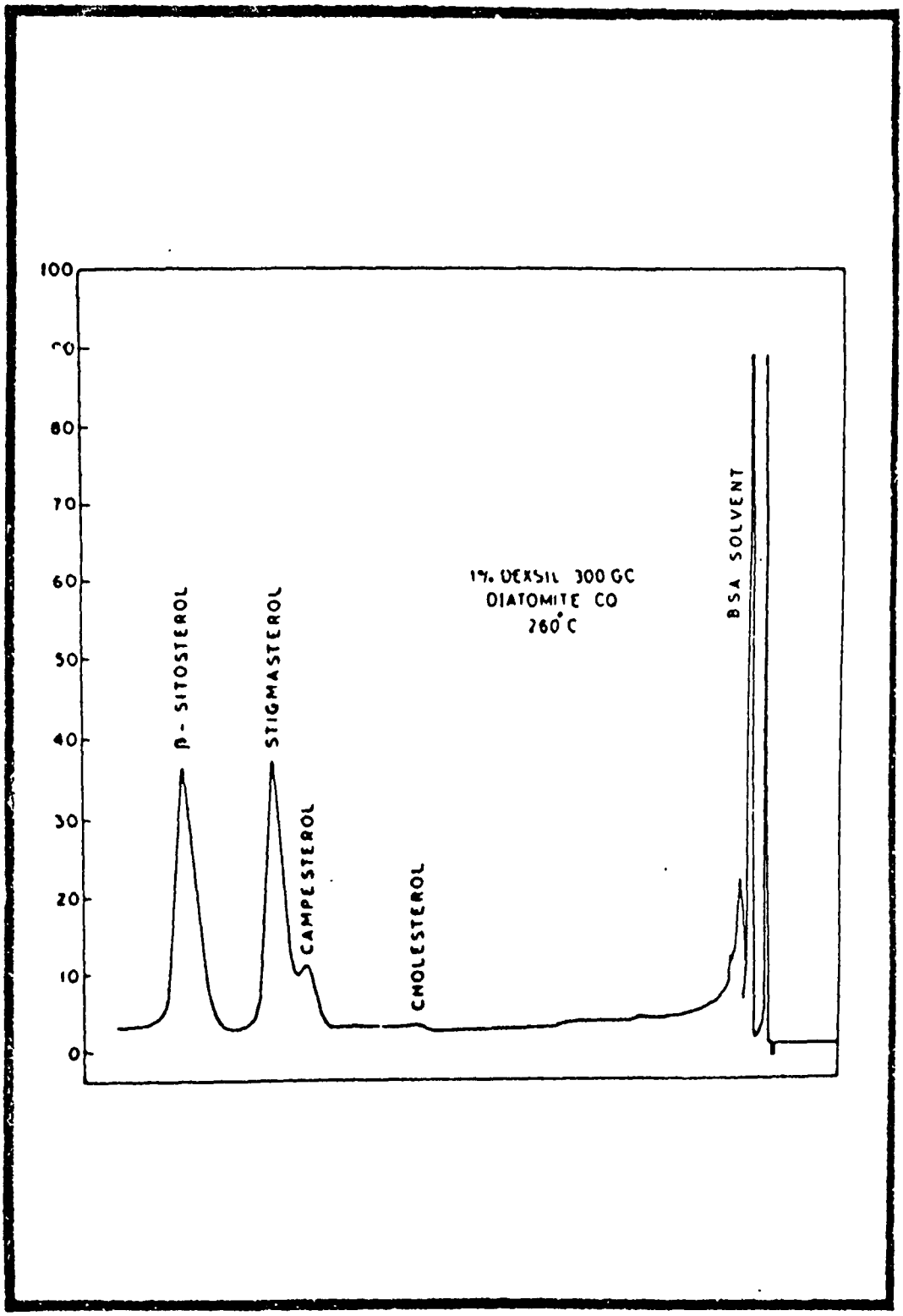
Mass spectral data of sterols as their trimethylsilyl ether

Mole Formula	High mass species* m/z (A%)	Identity
$C_{27}H_{45}OSiMe_3$	459 (9.8), 458 (24) $M^{+\cdot}$, 443 (12) $M^{+\cdot}$-15, 369 (17), 368 (68) $M^{+\cdot}$-90, 355 (9), 354 (11), 353 (36) $M^{+\cdot}$-90-15, 330 (20), 329 (100) $M^{+\cdot}$-129, 328 (26), 274 (19), 255 (22) $M^{+\cdot}$-S.C., 247 (15).	Cholesterol
$C_{28}H_{47}OSiMe_3$	472 (25) $M^{+\cdot}$, 457 (12) $M^{+\cdot}$-15, 383 (18), 382 (64) $M^{+\cdot}$-90, 368 (11) 367 (34) $M^{+\cdot}$-90-15, 344 (26), 243 (100) $M^{+\cdot}$-129, 342 (15), 389(5), 261(14), 255 (17) $M^{+\cdot}$-BC.S.C., 213 (13).	Campesterol (or 24epimer)
$C_{29}H_{47}OSiMe_3$	485 (25), 484 (59) $M^{+\cdot}$, 469 (15) $M^{+\cdot}$-15, 395 (24) 394 (68) $M^{+\cdot}$-90, 379 (25) $M^{+\cdot}$-90-13, 355 (36) $M^{+\cdot}$-129, 354 (19.8), 351 (43), 255 (100) $M^{+\cdot}$-90-S.C., 215 (23), 213 (27).	Stigmasterol (or 24 epimer)
$C_{29}H_{47}OSiMe_3$	488 (11), 486 (30) $M^{+\cdot}$, 471 (13) $M^{+\cdot}$-15, 397 (22), 396 (73) $M^{+\cdot}$-90, 382 (12) 381 (36) $M^{+\cdot}$-90-15, 358 (28), 357 (100), $M^{+\cdot}$-129, 356 (18), 275 (16), 255 (19)$M^{+\cdot}$-90-S.C., 213 (19).	β-sitosterol

*Only masses between m/z 650 and 205 are recorded as this is the most diagnostic region of the spectrum

Fig.-VII

Fig.-VIII


100
90
80
70
60
50
40
30
20
10
0
β - SITOSTEROL
STIGMASTEROL
CAMPESTEROL
CHOLESTEROL
1% DEXSIL 300 GC
DIATOMITE CO
260°C
BSA SOLVENT

GC–MS **Fig.-IX**

C_Y-5:

The product **C_Y-5**, m.p. 87^{0}C showed **ir** absorption υ^{kBr}_{max} at 2900, 1705, 1480, 1300, 940, 730 and 720 cm^{-1}, thereby indicating it to be an aliphatic carboxylic acid. Elemental analysis showed the molecular formula to be $C_{24}H_{48}O_2$, further confirmed by molecular ion peak at m/z 368. It gave methyl ester m.p. 58-59^{0}C. The compound was identified as **tetracosnoic acid**[11] **(III)**.

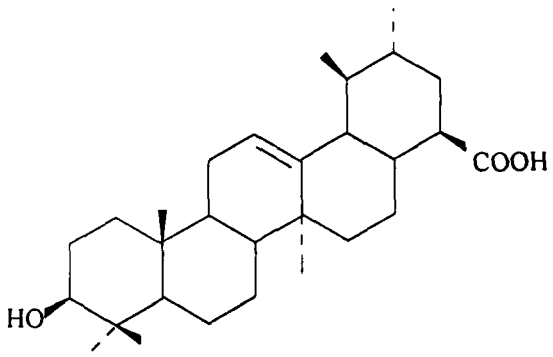
C_Y-6:

C_Y-6 on acetylation gave an acetate (C_Y-6A) m.p. 263-64^{0}C. The **ir** spectrum of the acetate showed absorptions at υ^{nujol} 1785, 1725, 1265 cm^{-1} characteristic of acetyl function.

The ^{1}**H-nmr** spectrum of the compound revealed seven-methyl groups at δ 0.8 (3H), δ 0.92 (3H), δ 1.0 (6H) and δ 1.1 (3H) and one acetoxyl at δ 2.1. In addition there was a triplet centered at δ 4.46 for a proton α- to the acetoxyl and a signal at δ 5.2 characteristic of the olefinic protons.

On methylation, C_Y-6A gave an acetyl methyl ester m.p. 236-37^{0}C. Its ^{1}**H-nmr** spectrum also showed methyl functions as signlets at δ 0.78 (3H), 0.9 (6H), δ 0.92 (3H) and δ 0.98 (3H) and one acetoxyl function as a signlet at δ 2.09. A singlet at δ 3.65 showed the ester methoxyl function. The olefinic proton signal was at δ 5.28 and a triplet for the proton α-to the acetoxyl at δ 4.46.

The acetate (C_Y-6A) on deacetylation gave the **genin** m.p. 268-70^{0}C. The above physical and spectral data of the genin and its derivatives showed that the compound is mono-hydroxy mono carboxylic acid. Its identify as **Ursolic acid (III)** was established by comparing the **ir** spectrum of its acetate with an authentic sample of ursolic acid acetate, which was super impossible.



(III)

C_Y-7:

TLC examination of **C_Y-7** m.p. 261-63^{0}C and its methyl ether m.p. 156-57^{0}C indicated it to be acacetin.[12] The **^{1}H-nmr** spectrum of C_Y-7-acetate **(Fig-X)** m.p. 204^{0}C showed two hydroxyl and one methoxyl groups. The **uv** spectrum of **C_1-7 (Table-3)** is comparable with the spectrum of acacetin and the **^{1}H-nmr** values of C_Y-7A and acacetin diacetate are recorded in **(Table-4)**.

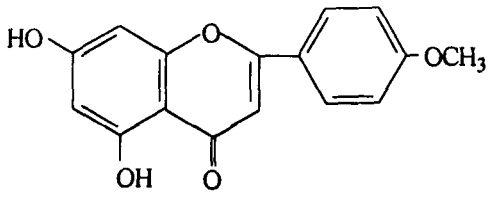
Table –3

UV Absorption spectra of C_Y-7 and acacetin (λ_{max}, nm)

Reagent	C_Y-7	Acacetin
MeOH	269, 303 SH, 327	270, 303 sh, 328
NaOMe	276, 295 sh, 383	275, 295 sh, 363
$AlCl_3$	259 sh, 277, 292 sh, 302, 344, 382	260 sh, 277, 291 sh, 301, 344, 381
$AlCl_3$/HCl	260 sh, 279, 294 sh, 300, 338, 379	260 sh, 280, 294 sh, 301, 337, 380
NaOAc	276, 297 sh, 358	276, 298 sh, 357
NaOAc/H_3BO_3	269, 309 sh, 331	269, 309 sh, 331

Band II in methanol has a peak at 269 nm and a pronounced infliction at 327 nm. $AlCl_3$ produces a 55 nm shift of Band I showing thereby a free 5-hydroxyl group. Sodium acetate, shifts Band II 31 nm indicating the presence of a free 7-hydroxyl group. The sodium acetate-boric acid spectra of C_Y-7 showed a blue shift (9 nm) in Band I relative to apigenin ($NaOAc/H_3BO_3$) with a decrease in its relative intensity, showing thereby a protected 4'-hydroxy group.

UV, m.p., and **^{1}H-nmr** spectra of C_Y-7A was found to be identical with that of acacetin diacetate **(Table-4)**, **C_Y-7** was, therefore, assigned the structure as **5,7-dihydroxy, 4'-O-methylflavone (IV)**.



(IV)

Table-4

Chemical shifts of protons of C_Y-7A and acacetin diacetate

Assignment	C_Y-7A	Acacetin diacetate
H-I-8	2.71 (1H, d, J=4 Hz)	3.45 (1H, d, J=3 Hz)
H-I-6	3.21 (1H, d, J=3 Hz)	3.80 (1H, d, J=2.5 Hz)
H-I-3	3.47 (IH, s)	3.63 (1H, s)
H-I-2', 6'	2.22 (2H, d, J=9 Hz)	2.20 (2H, d, J=9 Hz)
H-I-3', 5'	3.04 (2H, d, J=9 Hz)	3.05 (2H, d, J=9 Hz)
OMe/OAc:		
-4'	(6.12) (3H, s)	(6.14) (3H, s)
-5	7.62 (3H, s)	7.57 (3H, s)
-7	7.68 (3H, s)	7.67 (3H, s)

s = singlet, d= doublet, sepctrum run in $CDCl_3$ at 100 MHz, using TMS as internal standard = τ 10.00 Numbers in parentheses show chemical shifts of methoxy protons.

Fig.-X

C_Y-8:

C_Y-8 was eluted from column with benzene-ethylacetate (2:8, 1:9) mixture. The glycosidic nature of the product **(C_Y-8)** was evidenced by the positive Molish test obtained after hydrolysis. The glycosidic nature was further supported by the **^{1}H-nmr** spectrum of the acetate of C_Y-8A **(Table-5, Fig-XI)** as it showed two aromatic acetoxyls at δ 2.46 (3H) and δ 2.27 (3H) and four alcoholic acetoxyls at δ 1.99 (9H, s, 3-OAc), δ 1.73 (3H, s, OAc) indicating it to be a glucoside or galactoside.

The glycoside gave pink colour with Zn/HCl and red colour on treatment with sodium amalgam followed by acidification[13] indicating its flavone or flavanone nature. A yellow colour with Wilson boric acid reagent[14] and λ_{max} at 269 and 333 nm in the **uv** spectrum indicated it to be a flavone glycoside. It gave a brownish green colour with $FeCl_3$ indicating the presence of hydroxyl group at C-5. The **ir** spectrum displayed strong bands at 3400 cm^{-1} (OH) and 1700 cm^{-1} (C=O). A red shift of 15 nm with $AlCl_3$ further confirmed the presence of a free 5-OH group. No shift with fused NaOAc, ruled out the possibility of a free hydroxyl at 7-position.

The **^{1}H-nmr** spectrum of C_Y-8A **(Fig-XI)**, m.p. 100-11^{0}C, showed a sharp singlet at δ 6.48 indicating the presence of a C-3 proton of γ-pyrone nucleus. The presence of one methoxyl group was indicated through a singlet at δ 3.99. The remaining singlet in the spectrum was at δ 6.78 and it integrates for one hydrogen and can be assigned to an aromatic proton shielded by two ortho and one para oxygen and was found to arise from the C-8 proton of 5,6,7-trioxygenated flavone. The aromatic region showed a pair of two ortho coupled doublets integrating for two protons each centered at δ 7.78 (J=9 Hz) and δ 7.15 (J=9 Hz), attributed to an

A_2B_2 pattern. The shifts were therefore assigned due to 2',6' and 3',5'-protons of ring-B respectively.

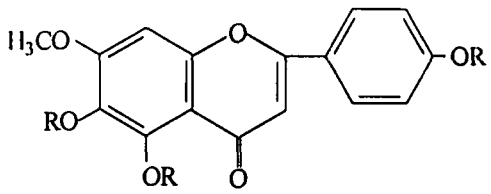
C_Y-8 on hydrolysis with 10% HCl gave an aglycone m.p. 290-92^{0}C **(V-a)**. The sugar was identified as glucose by R_f-values, co-chromatography with an authentic sample and by the formation of osazone.

Table –5

Assignment	**No. of protons**	**Signals**
H-3	1	6.48 (s)
H-8	1	6.78 (s)
H-2',6'	2	7.78 (d, J= 9Hz)
H-3',5'	2	7.15 (d, J=9Hz)
4 Aliphatic OAc of glucose moiety 2	12	1.73, 1.99 (s)
Aromatic OAc	3	2.27 (s)
Aromatic OAc	3	2.46 (s)
Aromatic OCH_3	3	3.99 (s)

s = singlet, d= doublet, spectrum run in $CDCl_3$, using TMS as internal standard (δ-scale).

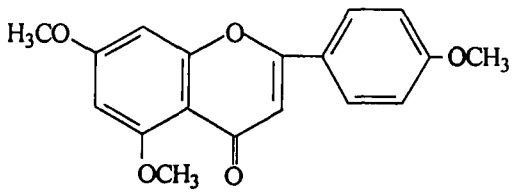
Demethylation of the aglycone with hydroiodic acid gave a yellow product, m.p. >350^{0}C, elemental analysis indicated to the molecular formula $C_{15}H_{10}O_6$. Acetylation of the compound with acetic anhydride and pyridine yielded a tetraacetate m.p. at 238-39^{0}C and showed no depression in melting point on admixture with an authentic sample of sceutellarein tetraacetate **(V-b)**. The aglycone **(V-a)** was therefore characterized as sorbifolin by ferric reaction. R_f values, spectral and chromatographic comparison with an authentic sample.[15]



(V-a), R = H
(V-b), R = Ac

On the basis of the above colour reactions and examination of the products of hydrolysis, the glycoside was identified as flavone glucoside having sorbifolin as an aglycone.

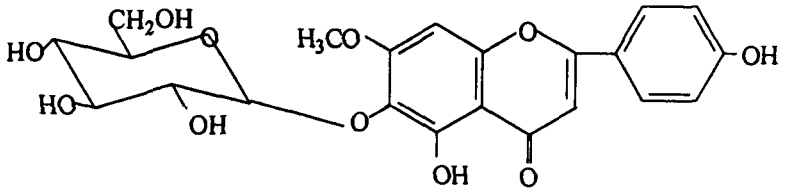
The position of the sugar residue in the glucoside was confirmed by the hydrolysis of the methylated glucoside. The partial methyl ether thus obtained was characterized as 6-hydroxy 4',5,7-trimethoxyflavone **(VI)** (m.p. 221^{0}C) by m.p. m.m.p. with an authentic sample[16] and **ultraviolet** spectral analysis with customary shift reagents.[17]



(VI)

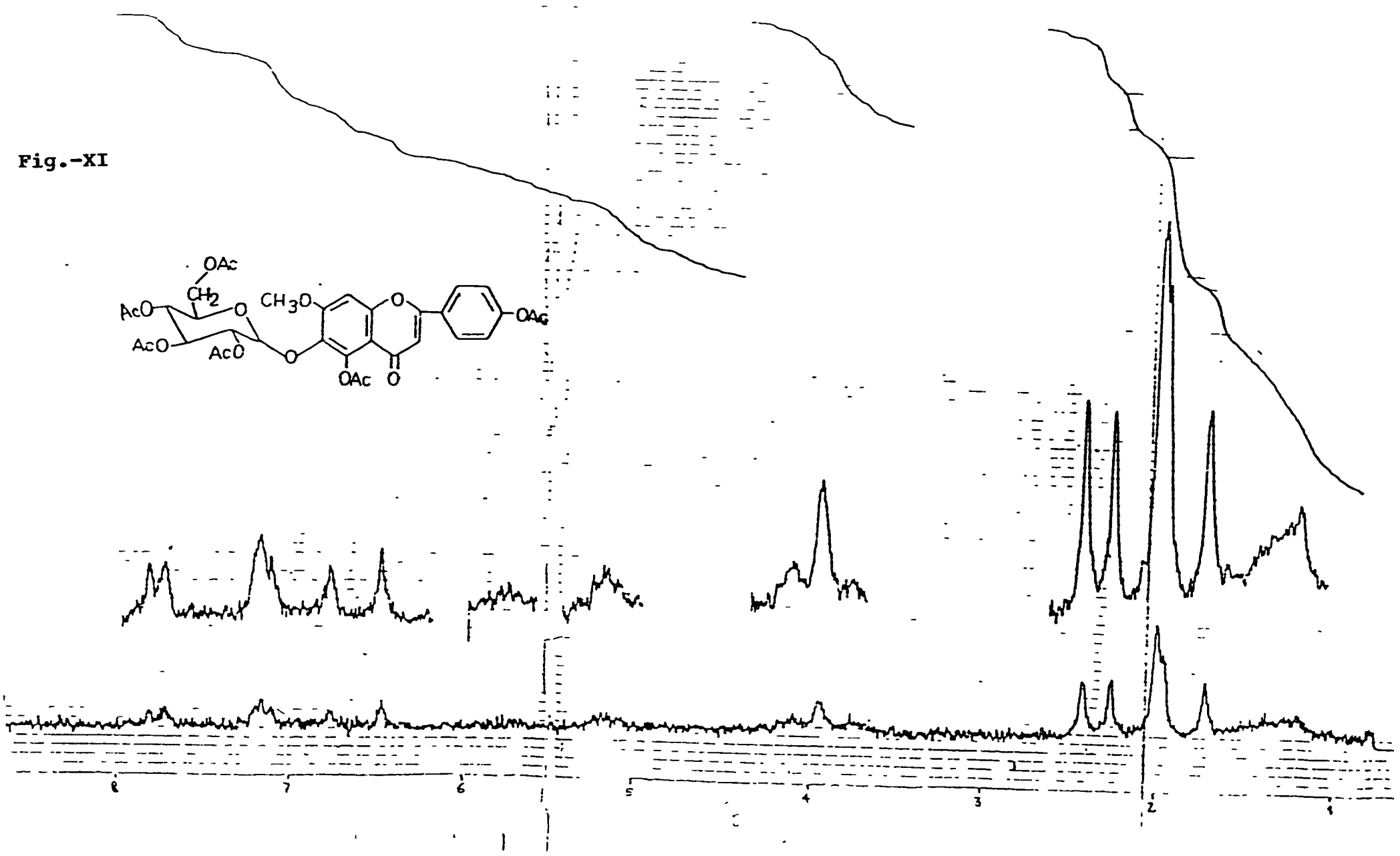
The quantitative estimation of sugar by Somogyis Copper micro method[18] showed the presence of one mole of glucose per mole of the aglycone.

C_Y-8 was therefore characterized as Sorbifolin 6-glucoside (VII).



(VII)

Fig.-XI



EXPERIMENTAL

STUDY OF THE BASE LEAVES OF CARYOTA URENS

Well dried and crushed base leaves (2 kg) were extracted successively with petroleum ether ($60\text{-}80^0$), benzene and methanol at room temperature and at their boiling points respectively. The petrol and benzene concentrates on TLC examination in petrol-ether as (4:1) solvent system showed at least six major spots, having the same R_f values. As the TLC behaviour of both the petroleum ether and benzene concentrates was the same, these two were combined (35 gm) together for further processing.

Separation into acidic and neutral part:

The dark green viscous mass (35 gm) was taken in ether, treated with aq. solution of potassium hydroxide (15%) and divided into alkali-soluble and alkali insoluble parts. The alkali insoluble part (25 gm) was refluxed with alcoholic potassium hydroxide (30 gm KOH dissolved in 600 ml of 80% ethanol) for half hour. Half of the solvent-was then distilled off and the contents were diluted with water (2 liters) and extracted three times with ether. All the ether extracts were combined together and washed with water, till free of alkali. The ethereal layer was dried over anhydrous sodium sulphate. Sodium sulphate was filtered off and ether was recovered to give the neutral part. (≈ 15 gm).

The aqueous layer was acidified with hydrochloric acid and extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulphate. The ether was removed, a light yellow solid (≈ 5 gm) was obtained.

Both the hot and cold extracts of methanol showed almost same spots on TLC examination in different solvent systems and therefore were mixed together and concentrated under reduced pressure the resultant mass was refluxed with petrol, benzene, chloroform, ethylacetate respectively and finally with acetone.

The ethylacetate and acetone concentrates on TLC examination in solvent system viz. TEF (5:4:1), BPF (36:9:5) and EtOAc: EtMeCO: AcOH: H_2O (2:3:1:1, 5:3:1) showed two compounds with varying concentration. They were combined and subjected to column chromatography over silica gel column using benzene-ethylacetate (9:1 to 1:1) yield two compounds **C_Y-7** and **C_Y-8** in different fractions. They were separated by repeated column chromatography.

Neutral part:

The neutral part (≈ 15 gm) was subjected to column chromatography over neutral alummina (1 Kg) and eluted with petrol, petrol-benzene and petrol-chloroform in different proportions and monitored by TLC. Following compounds **C_Y-1, C_Y-2, C_Y-3** and **C_Y-4** were isolated from different pools of identical fractions.

C_Y-1:

Elution of the column with petroleum ether (60-80^0) gave a dirty semi-solid mass. This was further purified by column chromatography over silica gel. On elution with hexane and repeated crystallization from carbon tetrachloride and acetone a colourless compound **C_Y-1** m.p. 62-67^{0}C was obtained. This showed an alongated spot on TLC (silica gel/$AgNO_3$, 5%) in petrol-benzene (4:1) solvent system. It was found to be a saturated hydrocarbon, (ir) and the elemental analysis, compared with that of triacontane; υ_{max} 2930, 2860 (C-H, saturated), 1460, 1380 (C-CH_3), 720 cm^{-1} $(CH_2)_n$.

Analysed for $C_{30}H_{62}$:

Calcd:	C, 85.30; H, 14.69%
Found:	C, 85.56; H, 14.58%

For final confirmation it was subjected to **glc** analysis which indicated this product to be a mixture of n-alkanes of the series C_{24}-C_{36} as given below **(Table-6)**. Odd number homologeus predominated as usual.

Table-6

S.No.	n-alkane		Composition %
1.	n-Triacontane	($C_{30}H_{62}$)	36.3
2.	n-Tri-Triacontane	($C_{33}H_{66}$)	28.9
3.	n-Non-acosane	($C_{29}H_{60}$)	21.2
4.	n-Triacontane	($C_{30}H_{62}$)	2.9
5.	n-Heptacosane	($C_{27}H_{56}$)	2.0
6.	n-Hexatriacontane	($C_{36}H_{74}$)	2.0
7.	n-Octacosane	($C_{36}H_{58}$)	2.0
8.	n-Hexacosane	($C_{26}H_{59}$)	trace ~~very small~~ quantity
9.	n-Pentacosane	($C_{35}H_{72}$)	trace ~~very small~~ quantity
10.	n-Pentatriacontane	($C_{36}H_{74}$)	trace ~~very small~~ quantity

C_Y-2:

Further elution of the neutral part with petroleum ether-benzene (1:4) and purification by repeated crystallization from methanol-chloroform gave a crystalline solid (**C_Y-2**), m.p. 214-15^{0}C, $[\alpha]^{20}_D$ + 23.64^0 ($CHCl_3$). It gave positive Lieberman-Burchard and Noller's test and yellow colour with tetranitromethane.

IR, υ^{kBr}_{max} cm^{-1}:

3360, 1030 (OH), 1645 (C=C), 1385 (germinal dimethyl), 885 (terminal methylene)

C_Y-2 was confirmed as **Lupeol** by m.p. and mixed melting point with an authentic sample. Further confirmation of the identity of the compound was obtained by spectral studies and by its derivatisation.

Acetylation of C_Y-2:

The compound (50 mg) was treated with acetic anhydride (2 ml) and pyridine (0.2 ml), allowed to stand overnight at room temperature and then heated on a water both for 6 hours. The solid product obtained, after usual work up, was crystallized from methanol-chloroform mixture as colourless flakes (60 mg), m.p. 218-20^{0}C.

IR, υ^{kBr}_{max} cm^{-1}:

875 (terminal methylene), 1245 (acetate), 1640 (C=C), 1730 (C=O).

^{1}H-NMR ($CDCl_3$) on δ scale:

0.82, .0.87, 0.94, 1.04, 1.27, 1.41, 1.46, 1.70, 2.03, ($OCOCH_3$), 2.28, 4.77 (>CHOAc).

Analysed for $C_{32}H_{52}O_2$:

Calcd.: C, 82.05; H, 11.11%

Found: C. 82.14; H, 11.17%

C_Y-3:

Elution of the column with benzene, followed by crystallization from benzene-ethylacetate gave a colourless amorphous powder, m.p. 259-60^{0}C. It gave a positive Stannic chloride test showing it to be a triterpene.

IR, υ^{kBr}_{max} cm^{-1}:

3415 (OH), 3060, 2940 (unsaturation), 2880, 1470, 1450, 1390, 1380 (characteristic of triterpenic skeleton), 1080, 1030 (C-O stretching and O-H in plane deformation of secondary alcohol), 815.

Mass, m/z:

$M^{+\cdot}$ 442, 424, 409, 339, 302, 287, 271, 257, 245 (100%), 220, 203, 202, 189.

Acetylation of C_Y-3:

The compound **(C_Y-3),** (100 mg) was treated with acetic anhydride (2.0 ml) and pyridine (1.0 ml) and left overnight at room temperature. After usual work up followed by crystallisation from ethanol colourless needles m.p. 245-47^{0}C, were obtained.

Mass, m/z:

$M^{+\cdot}$ 526, 511, ($M^{+\cdot}$-Me), 466 ($M^{+\cdot}$-HOAc), 344, 329, 284, 269, 262, 202, 189 (100%).

C_Y-4:

Elution of the column with benzene-chloroform (1:1) and purification of the product obtained from the column by repeated crystallization from methanol-chloroform afforded white crystalline solid **(C_Y-4)** m.p. 159-60^{0}C, $[\alpha]^{20}_D$ – 53.48^0. It gave positive Libermann-Burchard test and yellow colour with tetranitro-methane.

Analysed for $C_{29}H_{48}O$:

Calcd: C, 84.40; H, 11.72%

Found: C, 84.46; H, 11.91%

IR, υ^{kBr}_{max} cm^{-1}:

3350, 1050 (OH), 1655 (C=C), 840 (terminal methylene).

^{1}H-NMR, ($CDCl_3$) on δ scale:

0.70, 0.80, 0.88, 1.02 (CH_3 protons), 3.56 (3α-hydroxyl), 5.36 (1H, vinyl proton).

Acetylation of C_Y-4:

The above product (100 mg) was acetylated by the usual method, using acetic anhydride (2 ml) and pyridine (0.4). The acetate was crystallized from methanol-chloroform mixture as colourless flakes m.p. 126^{0}C.

IR, υ^{kBr}_{max} cm^{-1}:

1740 (C=O), 1680 (C=C), 1262 (acetate), 970 (terminal).

Benzoate:

C_Y-4, (50 mg) was dissolved in minimum amount of pyridine and benzoyl chloride (1 ml) was added to it. The mixture was heated over a boiling water bath for 8 hours, cooled to room temperature and poured into crushed ice with stirring. The solid obtained was washed with aqueous solution of potassium hydroxide (2%) followed by excess of water. The solid was crystallized from methanol, m.p. 144-45^{0}C (35 mg).

3,5-Dinitrobenzoate:

C_Y-4, (50 mg) was treated with freshly prepared 3,5-dinitrobenzoyl chloride (60 mg) and pyridine (0.5 ml) and heated over a water bath for 45 mints. After usual work up the crude derivative was crystallized from acetone and methanol m.p. 208-12^{0}C.

Finally **gc-ms** analysis of the sterol (TMS derivative) indicated it to be a mixture of cholestrol ($M^{+\cdot}$ 458), campesterol ($M^{+\cdot}$ 472, m/z 457, 383, 382, 368, 344, 255, 213 etc.), stigmasterol ($M^{+\cdot}$ 484, m/z 469, 395, 394, 379, 355, 354, 255, 215, 213 etc) and β-sitosterol ($M^{+\cdot}$ 486, m/z 471, 397, 396, 382, 357, 275, 255, 213 etc.).

Alkali soluble part:

The yellow acidic part (≈ 5 gm) was dissolved in benzene-ether (8:1, v/v) and chromatographed over silica gel (300 gm). Mainly two products were obtained marked as **C_Y-5** & **C_Y-6**.

C_Y-5:

On elution with petroleum ether (60-80^0) **C_Y-5**, m.p. 87^{0}C was obtained. This appeared to be a saturated (negative tetranitromethane test) aliphatic acid υ_{max} 2900, 1300, 940 (OH), 1705, 1480 and 730, 720 cm^{-1} $(CH_2)_n$.

The methyl ester of **C_Y-5** was prepared by treatment with absolute methanol. On crystallization with acetone, low melting (58-59^{0}C) crystals were obtained.

C_Y-6:

Further elution of the column with ethylacetate, a colourless product was obtained. It was crystallized from chloroform-methanol as colourless shining needles, m.p. 284-88^{0}C. It appeared to be ursolic acid on the basis of its m.p., m.m.p. and co-TLC with an authentic sample. Its identity as **Ursolic acid** was further supported by derivitisation of the **C_Y-6**.

Acetylation of C_Y-6:

Acetate was prepared by usual method, m.p. 263-64^{0}C. It showed no depression in melting point when mixed with an authentic sample of ursolic acid acetate.

IR, υ^{nujol}_{max} cm^{-1}:

1785, 1725, 1265 .

^{1}H-NMR ($CDCl_3$), on δ scale:

0.8 (3H, s), 0.9 (s), 0.92 (3H, s), 1.0 (6H, s), 1.1 (3H, s), 2.1 (s), 4.46 (t), 5.26 (m).

Mass, m/z:

498 ($M^{+\cdot}$), 438, 249, 203, 189.

Acetyl methyl ester:

The above compound was treated with diazomethane. After usual work up followed by crystallization from methanol gave colourless needles m.p. 236-37^{0}C.

^{1}H-NMR ($CDCl_3$) on δ scale:

0.78 (3 H, s), 0.9 (6H, s), 0.92 (3H, s), 0.98 (3H, s), 0.98 (s), 2.09 (s), 3.65 (s), 4.46 (s), 5.28 (m).

Mass, m/z:

512 ($M^{+\cdot}$), 452, 262, 249, 203, 184.

C_Y-7:

C_Y-7 (60 mg) was refluxed with dimethyl sulphate (0.7 ml), anhydrous potassium carbonate (1 gm) in dry acetone (100 ml). After usual work up a methyl ether corresponding to trimethyl ether of apigenin (55 mg) m.p. 156-57^{0}C was obtained.

5,7-Diacetoxy, 4'-O-methylflavone (C_Y-7A):

C_Y-8 (100 mg) was acetylated with pyridine and acetic anhydride. On crystallization from $CHCl_3$-MeOH colourless needles m.p. 204^{0}C (80 mg) were obtained.

^{1}H-NMR ($CDCl_3$) on δ scale:

2.71 (1H, d, H-I-8), 3.21 (1H, d, H-I-6), 3.47 (1H, s, H-I-3), 2.22 (2H, d, H-I-3',6'), 3.04 (2H, d, H-I-3',5'), 6.12 (3H, s, OMe-I-4'), 7.62, 7.68 (s, 3H, each, OAc-5,7 respectively).

UV with shift reagents, λ_{max}, nm:

MeOH	269, 303 sh, 327
NaOMe	276, 295 sh, 383
$AlCl_3$	259 sh, 277, 292 sh, 302, 344, 382
$AlCl_3$/HCl	260 sh, 279, 294 sh, 300, 338, 379
NaOAc	276, 297 sh, 358
NaOAc/H_3BO_3	269, 309 sh, 331.

C_Y-8:

C_Y-8 was crystallized from ethylacetate-methanol as yellow needles, m.p. >310^{0}C.

Analysed for $C_{22}H_{22}O_{11}$:

Calcd.: C, 57.14; H, 4.76%

Found: C, 57.20; H, 4.80%.

UV with shift reagents, λ_{max} nm:

MeOH	269, 333
$AlCl_3$	280, 290, 348, 375 sh
$AlCl_3$/HCl	280, 291, 348, 375 sh
NaOAc	269, 375
NaOAc/H_3BO_3	271, 334
NaOMe	370, 388

IR, υ^{kBr}_{max} cm^{-1}:

3400 (OH), 1700 (C=O).

Acetylation of C_Y-8:

A crystalline glycoside (35 mg), was heated with acetic anhydride (3 ml) and dry pyridine (1.5 ml) at 100^{0}C for 3 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The separated solid was filtered, washed well with water and dried. On crystallization from dilute ethanol it gave colourless crystals (25 mg), m.p. 110-11^{0}C.

^{1}H-NMR ($CDCl_3$) on δ scale:

6.48 (1H, s, H-3), 6.78 (1H, s, H-8), 7.15 (2H, d, J=9 Hz, H-3',5'), 7.78 (2H, d, J= 9 Hz, H-2',6'), 3.99 (3H, s, OCH_3-7). 2.27 (3H, OAc-4'), 2.46 (3H, OAc-5'), 1.99 (9H, s, 3 x OAc), 1.73 (3H, s, OAc).

Acid hydrolysis of C_Y-8:

The glycoside (100 mg) was dissolved in 25 ml of 10% aqueous HCl-MeOH (1:1) and heated on a water bath. The hydrolysis appeared to be completed within 30 minutes. The heating was continued for two hours to ensure complete hydrolysis. After leaving overnight, the yellow aglycone thus separated out was filtered, washed well with water and dried. The crude product on crystallization from methanol gave yellow needles (70 mg), m.p. 290-92^{0}C and showed no depression on admixture with an authentic sample of sorbifolin.

Analysed for $C_{16}H_{12}O_6$:

Calcd.: C, 64.00; H, 4.00%

Found: C, 63.96; H, 3.98%.

UV with shift reagents, λ_{max} nm:

MeOH	253, 308
$AlCl_3$	263, 323
NaOAc	254, 309

IR, υ^{KBr}_{max} cm^{-1}:

3260, 1655,

Acetylation of Sorbifolin:

Sorbifolin (25 mg) was heated under reflux with acetic anhydride (2.5 ml) and fused sodium acetate (100 mg) on a water bath for two hours. After cooling, the mixture was poured over crushed ice and left overnight. The solid was collected, washed with water and dried, on crystallization from ethanol it gave clolurless needles (18 mg), m.p. 238-39^{0}C.

Analysed for $C_{22}H_{18}O_9$:

Calcd.: C, 61.95; H, 4.26%

Found: C, 62.03; H, 4.30%.

^{1}H-NMR ($CDCl_3$) on δ scale:

7.90 (2H, d, J=9 Hz, H-2',6'), 7.28 (2H, d, J=9 Hz, H-3',5'), 6.60 (1H, s, H-8), 6.58 (1H, s, H-3), 3.80 (3H, s, OCH_3-7). 2.42 (3H, s, OAc-5), 2.35 (6H, s, OAc-4',6).

Methylation of Sorbifolin:

Sorbifolin (20 mg), dimethyl sulphate (0.5 ml), anhydrous potassium carbonate (1.0 gm) and acetone (100 ml) were refluxed for 24 hours. The reaction

mixture was filtered and the residue washed several times with hot acetone. On distilling off the solvent, a brown viscous semisolid mass was left behind. It was washed with hot petroleum ether to remove the excess of dimethyl sulphate. The solid residue on crystallization from ethylacetate-methanol gave colourless needles (10 mg), mp. 188-89^{0}C.

Analysed for $C_{19}H_{18}O_6$:

Calcd.: C, 66.66; H, 5.26%

Found: C, 66.61; H, 5.23%.

Chromatographic identification of sugar:

The acidic filtrate, left after filtering the aglycone was extracted with ether and then with ethylacetate to ensure the complete removal of the aglycone. The solution was concentrated to a syrup in vacuum over NaOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on Whatman No. 1 filter papers using butanol-acetic acid-water (4:1:5) and butanol-water-ethanol (60:28.5:16.5) as solvent systems, employing descending techniques. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature were sprayed with aniline pthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05^{0}C showed the presence of only glucose.

Estimation of sugar:

The anhydrous glycoside (20.5 mg) was hydrolysed by refluxing for two hours with 2% H_2SO_4. After cooling overnight, the aglycone was filtered, washed, dried and weighed (13.2 mg). Thus the ratio to the glycoside is 64.3% and this ratio indicates the presence of one mole of sugar per mole of the aglycone.

The quantitative estimation of sugar by Somogyis copper micro method gave the value (0.44 ml) which corresponds to 1 mole of sugar / mole of aglycone.

6-Hydroxy-4',5,7-trimethoxyflavone:

Glycoside (30 mg) was dissolved in dry acetone and refluxed with an excess of dimethyl sulphate (1.2 ml) and fused potassium carbonate (3 gm) for 36 hours on a water bath. The mixture was filtered and the residue was washed with hot acetone. After distilling off the solvent from the filtrate brown residue was ~~left behind~~ obtained. The excess of dimethyl sulphate was removed by washing the methylated product several times with hot petroleum ether. It was hydrolysed by heating with 7% H_2SO_4 for two hours. The reaction mixture was left overnight, a faintly yellowish powder separated out. It was filtered washed with water and dried. On several crystallization from methanol it gave straw needles (16 mg), m.p. 221^{0}C.

Analysed for $C_{18}H_{16}O_6$:

Calcd.: C, 65.85; H, 4.87%

Found: C, 65.81; H, 4.82%.

REFERENCE

1. '**The Wealth of India**,' Raw Material, CSIR, Publication, New Delhi, Vol. I, p.90 (1948).

2. **Indian Material Medica**, A.K. Nadkarni, Vol.I, p.280 (1954).

3. Nambiar, M.K. Geetha, Shafi, P. Mohan **Asian J. Chem., 8(4)**, 563-564 (1964)

4. I. Rabarisoa, E.M. Gaydou, J. P. Bian Chini, **Oleagineux, 48(5)**, 25-5 (1993).

5. N. Gopinathan, **Intern. Sugar J., 64**, No.757, 9-11 (1962).

6. K. Cell, Stranksy, Czech, **Chem. Comm., 32**, 3215 (1967); **37**, 4106 (1972).

7. C.R. Noller, R.A. Sonith, G.H. Harris and J.W. Walker, **J. Amer, Chem. Soc. 64**, 3027 (1962).

8. J.S. Chauban and S.K. Srivastava, **Phytochemistry, 17**, 1005 (1978).

9. H. Budzikiewiez, J.M. Wilson and C. Djerassi, **J. Amer. Chem. Soc., 85**, 3688 (1963).

10. C.J.W. Brook, E.C. Horning and J.S. Young, **Lipids, 3**, 391 (1968).

11. L. Prakash and Mrs. Gitagarg, **J. Ind. Chem. Soc., L VIII**, 726 (1981).

12. E. Rodriguez, N.J.Carman, G. Vander Velde, J.H. Mereynholds, T.J. Mabry, M.A. Irwin and T.A. Geissman, **Phytochemistry, 11**, 3509 (1972).

13. K. Venkataraman, Fortsch, **Chem. Org. Nat., 17**, 1 (1959)

14. C.W. Wilson, **J. Amer. Chem. Soc., 61**, 2303 (1939).

15. M. Arisawa, T. Takakuwa and T. Nakaoki, **Chem. Pharm. Bull.** Tokyo, **18,** 916 (1970).

16. L. Farkas, M. Nagradi, V. Sudarsanam and W. Herz, **J. Org. Chem., 31,** 3228 (1966).

17. T.J.Mabry, K.R. Markham and M.B. Thomas, **'The Systematic Identification of Flavonoids'**, Springer, New York (1970).

18. M. Somogyis, J. **Biol. Chem., 195**, 19 (1952).